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A WIDE RANGE VACUUM TUBE VOLTMETER CIRCUIT[1]

BY G. A. WOONTON[2]

Abstract

The conventional type of vacuum tube voltmeter has been found to be unsatisfactory when used to check amplifiers designed to reproduce e.m.f's whose fundamental frequencies range from a few to 100 cycles per second. These difficulties can be overcome by the use of a symmetrical tube circuit which integrates the two halves of the input wave to produce a smoothed direct current component. Certain other advantages which result from the use of the same circuit are reported. The choice of Type 31 tubes makes the use of an inexpensive plate current meter possible.

Introduction

This investigation was undertaken as part of a general program intended to facilitate the application of physical equipment to medical research.

The application of vacuum tubes to the investigation, recording, and reproduction of an ever increasing number of phenomena has led to the design and construction of amplifiers intended to satisfy a wide variety of conditions. In all cases, amplifiers may be checked and calibrated with the greatest facility, through the medium of the vacuum tube voltmeter.

The conventional type of vacuum tube voltmeter has been found to be unsatisfactory when used to measure the output of amplifiers designed to operate at very low frequencies, or to reproduce exceedingly asymmetrical wave forms. In many cases these amplifiers are used to drive some type of oscillograph, the input to which usually ranges from a few volts up to 100 volts. Since reflexing destroys the square law characteristic of a vacuum tube voltmeter, *i.e.*, introduces wave form error, it is desirable that the voltmeter have a sufficiently extended square law characteristic to allow convenient multiplication of the range up to this value by means of some series resistor arrangement.

[1] *Manuscript received October 1, 1934.*

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Description of the Circuit

With the voltmeter circuit shown in Fig. 1, it is possible to check the performance of amplifiers of widely varying design.

Certain advantages are gained by the symmetrical arrangement of circuit elements. Since only the direct current component of detection flows in the meter circuit, the A-C. components being 180° out of phase, filters and bypasses, which must inevitably introduce both wave-form and frequency error at low frequencies, are not required.

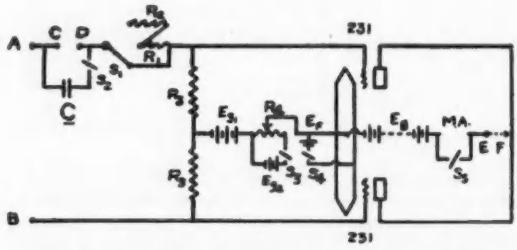

FIG. 1. *The voltmeter circuit. AB, input terminals; CD, terminals for bucking battery circuit; EF, terminals for exterior current meter; C, 2 microfarads; S_1, range switch; $S_2S_3S_4S_5$, switches (S_3 and S_4 ganged); R_1, 1 megohm; R_2, 3 megohms; R_3, 0.25 megohm; R_4, 2000 ohm variable potential divider; E_{g1}, 12.5 volts; E_{g2}, 1.5 volts; E_F, 1.5 volts; E_B, 45 volts; M.A., 1 milliampere range, Weston model 301 d.-c., milliameter.*

The symmetrical input system fixes the zero axis of the applied input voltage at the cathode potential. The voltmeter automatically integrates the two halves of the input wave, regardless of asymmetry, to give the true effective value.

Since the two grid circuits are effectively in series, the voltmeter will have a square law characteristic over twice the range of applied voltage for a single tube, and at the same sensitivity, since the direct current components of detection are additive in the meter circuit.

It is common practice with all types of effective value tube voltmeters to set the tube bias to give a false zero of plate current. A high value of initial current reduces the percentage error in setting the bias because of the lower percentage error in reading the plate current, but at the same time disproportionately reduces the range over which the tube operates as a square law detector. The circuit illustrated in Fig. 1 gives the same accuracy in setting the bias, for half the plate current per tube, as would be necessary in a single tube circuit, since the plate currents from the two tubes are additive.

The input resistance of the symmetrical circuit is double that for a single tube voltmeter, or viewed in a different way, the resistance in the grid circuit of each tube is just one-half that necessary in a single tube voltmeter to give the same input resistance. A reasonably low grid circuit resistance is important in a voltmeter to be used on either open or short circuited input. It should also be noticed that even on short circuit and for the unmultiplied range the grid circuit resistance can reduce to only one-half of its open circuit value.

Precision

The voltmeter may be calibrated conveniently at 25 or 60 cycles per second, and used to measure potential differences whose fundamental com-

ponents have frequencies as low as 10 cycles per second without special precautions except the introduction of condensers of from 2 to 4 μfd across *CD*. For frequencies between 2 and 15 cycles per second a bucking battery circuit is substituted for the input condenser, if a direct current component flows in the circuit to be measured, and a well damped meter of long period placed across *EF*, the milliameter being shorted out to prevent damage from mechanical shocks.

Since the voltmeter reads the effective value of the applied potential difference, without regard to the sense of the various components, the voltmeter reading must increase when either the bucking battery potential, or the applied direct current component, predominates. The value of the alternating component may be conveniently found by manipulating the bucking battery potential to give a minimum reading on the plate current meter.

It was found by experiment that compensating errors appeared when a false zero current much larger than 0.02 milliamperes was used. With this setting a device for balancing out the zero current is not required. The precision of the instrument was found as:—

6–10 volts, $\pm$ 0.1 volts	2–3 volts, $\pm$ 0.25 volts
3– 6 volts, $\pm$ 0.2 volts	below 2 volts, error extremely large.

On the multiplied scales these errors increase in the multiplier ratio.

These data include errors due both to the operator's inability to read the small panel type self-contained meter closer than 0.01 ma. and to the lack of precision in setting the false zero due to the same cause. Errors entering into the calibration because of lack of precision in the calibrating instruments must be added to these values.

Fig. 2 shows the calibration curve for the instrument, and the precision that may be expected over each of its ranges, including the error introduced in calibrating. These precisions may be somewhat improved if a more accurate current meter is employed in the plate circuit.

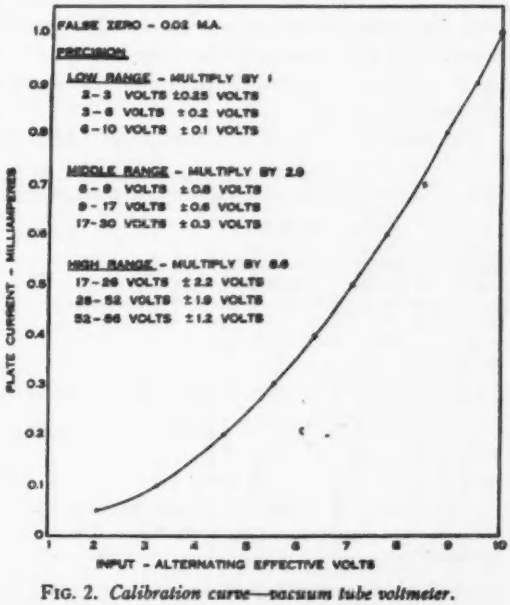

FIG. 2. *Calibration curve—vacuum tube voltmeter.*

Fig. 3 is a plot of plate current against the square of the applied effective volts and shows how accurately the meter follows the desired square law characteristic.

It was found that a uniform drop of ¼ of 1% in calibration could be expected per 1% drop in plate battery potential between 50 and 40 volts. Since the circuit is subjected only to intermittent use, the calibration may be expected to hold accurately for many months. The calibration of the instrument changes very rapidly with changes in filament potential. The filaments of two 231 tubes, connected in parallel as in this circuit, draw approximately 200 ma. at 1.5 volts. It is recommended that two dry cells of standard size be used in parallel to supply the filament current, in order to minimize error from this source. Since the bias is set, by a plate current reading, no error appears in the calibration because of shifts in grid battery potential.

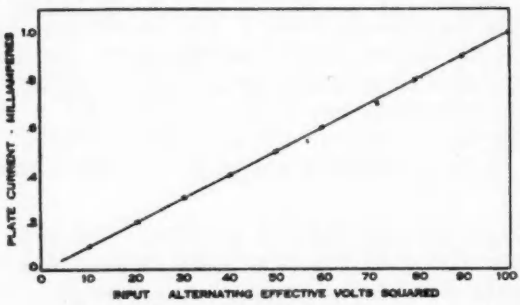

FIG. 3. *Vacuum tube voltmeter calibration curve (exhibiting square law characteristics).*

In order to determine wave form, frequency and turn over error, the meter was calibrated from the commercial mains, which supplies current at 25 cycles per second and whose wave form is a much flattened sinusoid. The meter was then used to measure potential differences across a known resistor due to square waves generated by commutating direct current. The form could be controlled so as to obtain either square unidirectional pulses or a true alternating current.

The results in Table I are typical. They were obtained by holding the effective voltage across the voltmeter constant, while varying the frequency. The voltages employed had a symmetrical, square wave form.

TABLE I

Frequency, c.p.s.	Volts from panel meter	Volts from exterior high inertia meter	Remarks
50	9.8	9.8	
24.5	9.8	9.8	
9.5	9.7	9.7	
1.2	—	9.6	High inertia meter shows ripple of about 2% full scale
50	7.0	7.0	
24.5	7.0	7.0	
9.5	7.0	7.0	
1.2	—	6.9	Ripple approximately 1% of full scale

Turn over and wave form errors were not apparent at any time, even for unidirectional pulsating potential differences.

The ripple which appears in the high inertia meter at these low frequencies has been found to reduce the precision with which the meter may be read to about the same value as that found for the Weston milliameter.

Although this meter has not been checked at high frequencies, its design suggests that it should give excellent results throughout the whole range of audio frequencies and well up into the radio frequency band.

Exceedingly asymmetrical wave forms containing very low frequency components are most commonly met in the investigation of physiological phenomena through their electric effects, such as the e.m.f's accompanying cardiac action, or nervous impulses. In some cases involving the amplification of speech sounds, serious errors in measurement occur through the inability of the ordinary vacuum tube voltmeter to compensate for asymmetry. Although this voltmeter finds its most advantageous application in the cases just mentioned, it has in addition the same range of application as all other types of tube voltmeters, since no new disadvantages are introduced.

The relatively high plate current made possible by the use of the Type 31 tubes allows the use of an inexpensive type of plate current meter. Cheapness, combined with several of the other features enumerated, should make this type of voltmeter especially valuable to the radio amateur.

THE EXCITATION OF BAND SPECTRA—ROTATIONAL STRUCTURE[1]

By G. O. Langstroth[2]

Abstract

An examination of the intensity contours of three second positive nitrogen bands excited by electrons of 14, 15, 16 and 18 electron volts energy, indicates that the contours change in shape as the energy of the exciting electrons is varied. These results and their relation to those of other investigators can be understood if there is a definite probability that an impinging electron will excite the electronic configuration of a molecule and then interact with the rotation before escaping from the molecular field. As might be expected, this probability is appreciable only when the energy of the exciting electron is nearly equal to the excitation energy.

The intensity ratios of the second positive nitrogen bands have been shown to be independent of the energy of the exciting electrons for energies between 25 and 160 electron volts, and the change for energies between 14 and 25 electron volts, if any, is slight (4, 9). It would appear to follow that interchange between electronic translational energy and molecular vibrational energy is improbable under these conditions.

The writer has examined the intensity contours of the bands on his plates in order to see whether there is any evidence of interchange between electronic translational energy and molecular rotational energy, *e.g.*, a dependence of the shape of the band contours on the energy of the exciting electrons. Such a dependence does occur for the bands 0→2 (λ3805), 1→3 (λ3755), and 2→4 (λ3710), for exciting voltages of 14, 15, 16 and 18 volts. The tail intensity is greater, relative to the maximum intensity, the lower the voltage. The change takes place in the same general way for each band.

An approximate analysis of the results indicates that at the lowest exciting voltage the populations of the higher rotational levels are relatively greater than they would be in thermal equilibrium, but that as the voltage is increased they become more nearly those expected for a Boltzmann distribution. Other investigators (7, 8), using similar excitation tubes but higher exciting voltages, have consistently found a Boltzmann distribution in the initial levels.

The observed changes in contour must be connected either with the changes in filament temperature which are necessary to maintain constant tube current as the voltage is varied, or with a direct dependence of the initial level populations on the energy of the exciting electrons. Since there are reasons to believe that the former cannot explain the results, one must look to the latter for the probable explanation.

These results and their relation to the results of others at higher exciting voltages could be understood if a double switch takes place between the energy of the exciting electron and the electronic and rotational energy of

[1] *Manuscript received October 25, 1934.*
Contribution from the Physics Department, McGill University, Montreal, Canada.
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the molecule. This must occur relatively frequently only when the energy of the electron is nearly equal to the excitation energy. Such a process appears to be probable from a consideration of the energy relations.

Some measurements of the intensities of rotation lines with exciting voltages of from 14 to 20 volts and with the proper precautions to eliminate extraneous effects would determine the validity of this explanation, and might lead to some very interesting results.

Experimental

The excitation tube, which consisted essentially of an oxide-coated platinum filament, a guard ring, and a cage with a grid at the filament end, was filled to a pressure of 0.1 mm. of mercury with purified nitrogen, and was operated with tube currents of 0.30 ma. The spectrograph slit was opened to 0.5 mm. to blend the rotational structure completely. Details of the technique are given in former articles (4, 9).

The band contours were determined by measuring the intensity at about 25 points on the microphotometer records for each band by the methods of reference (4, 9). An analyzing apparatus (15) was originally used to do this and later, as a check, straightforward distance measurements were made on new microphotometer records. There was no difficulty in determining corresponding plate distances on the various contours in arbitrary units, and fortunately the change of intensity with plate distance was not great near the tail. No background or interfering bands or lines were present. The contour of λ3805 was determined for exciting voltages of 14, 15, 16 and 18 volts, and two examples are shown in Fig. 1. Measurements were also made at selected points on the contours of λ3805, λ3755, and λ3710 on other plates. Individual relative intensity measurements should be good to within 10%.

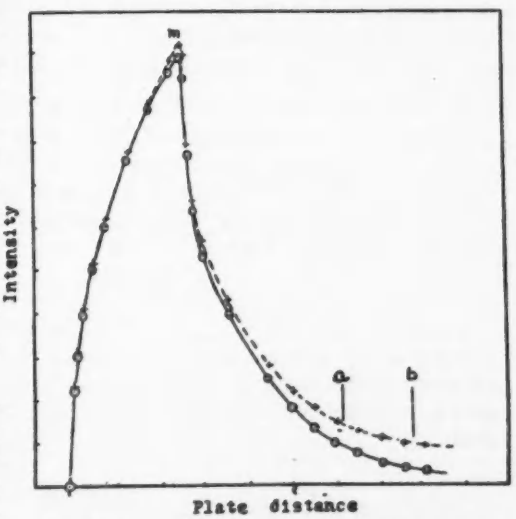

FIG. 1. *Comparison of the contour of λ3805 at 18 volts (solid line), and at 14 volts (broken line) accelerating potential. The intensity scale of the former is four times that of the latter. The points represent measured intensities. The plate distances are in arbitrary units which are the same for both contours.*

An approximate analysis of the contours can be made if the group of rotational lines responsible for the contour intensity at any point is known. To find it one must determine the broadening (to the violet) of the individual rotation lines

due to the wide slit, their contour, and the frequency which any point on the observed contour would represent if a narrow slit had been used. The broadening was determined from comparator measurements of the width of the mercury lines λ3650 and λ4046 which appeared faintly on the plates. The contours of these lines were flat topped. The "narrow slit" frequencies were determined from measurements on the band edges and the red side of the mercury lines. It was then possible from a consideration of the Fortrat diagram of the rotational structure (2, 5, 6) to assign to each point on the contour the sum of the intensities of certain rotational lines.

Three points on the contour of λ3805 were chosen—the maximum which contained $R(0)$ to $R(12)$ and $P(1)$ to $P(27)$, Point a which contained $R(19)$ to $R(25)$ and $P(34)$ to $P(40)$, and Point b which contained $R(24)$ to $R(29)$ and $P(38)$ to $P(43)$. $R(n)$ and $P(n)$ include all members of the triplet and Λ-type doublet structure. The intensities of these points will be referred to as I_m, I_a, and I_b.

It was possible to check the allotment of lines in the maximum by calculation of the intensity for different allotments, assuming a Boltzmann distribution in the initial levels. For temperatures in the range under consideration the calculated position of the maximum agreed closely with that found from the plate measurements. This value is very critical as may be judged from the rapid intensity change on either side of it. The allotments for Points a and b are more easily made owing to the wider separation of the rotation lines in this region.

Contour Analysis

The relative intensities of individual rotation lines can be calculated from their transition probabilities and the distribution of molecules in the initial states. The intensity factors (transition probability multiplied by statistical weight) of the R and P lines (considered as "unresolved" triplets) were taken as approximately those given by the Hönl-London formulas for a Type a molecule (14), *i.e.*,

$$\bar{R}(n) = A(\kappa-1/\kappa)\,; \quad \bar{P}(n_1) = A(\kappa+2)\kappa/(\kappa+1), \tag{1}$$

where κ is the rotation quantum number of the *initial* level, and the line numbers n and n_1 are equal to $(\kappa - 1)$ and $(\kappa + 1)$. If the initial level distribution is a Boltzmann distribution as many experimenters have found, it is given by,

$$N(\kappa) = N(o)\ .\ (2\kappa+1)e^{-\frac{B\kappa(\kappa+1)}{kT}} \tag{2}$$

where $N(\kappa)$ and $N(o)$ denote the relative numbers of molecules in the κ and in the lowest level, k the Boltzmann constant, T the distribution temperature, and B the rotation constant which is 1.814 cm.$^{-1}$ for λ3805 (3).

I_m, I_a, and I_b are then approximately,

$$\left.\begin{aligned} I_m &= A\left[\sum_{n=0}^{19}\bar{R}(n)\,.\,F(K)+\sum_{n=1}^{21}\bar{P}(n)\,.\,F(K)\right] \\ I_a &= A\left[\sum_{n=19}^{25}\bar{R}(n)\,.\,F(K)+\sum_{n=26}^{40}\bar{P}(n)\,.\,F(K)\right] \\ I_b &= A\left[\sum_{n=26}^{39}\bar{R}(n)\,.\,F(K)+\sum_{n=40}^{42}\bar{P}(n)\,.\,F(K)\right] \end{aligned}\right\} \quad (3)$$

where $F(K) = N(K)/(2K+1)$, and the usual relations between n and K hold.

If a Boltzmann distribution holds, the distribution temperature for a contour can be determined from the measured intensity ratio of, for example, I_m and I_a, and the theoretical curve for the variation of this ratio with temperature (Equations (1), (2) and (3)). The three temperatures which can be obtained from the three measured intensities should agree if the assumption of a Boltzmann distribution is correct. If there are large discrepancies it is possible to obtain an estimate of the direction and magnitude of the variation of the actual distribution from a Boltzmann distribution.

The following values for a temperature of 600°K give an idea of the magnitude of the terms on the right-hand side of Equations (3). The first and second terms in the first, second, and third equations are respectively, 58.5, 116; 15.2, 0.9; 5.9, 0.1. I_m may be taken as characteristic of the populations of low levels since the strongest lines at 600°K are $R(10)$ and $P(11)$, and I_a and I_b are predominantly influenced by the populations of narrow regions from $K = 20$ to 26, and $K = 25$ to 30 respectively.

Results

The values in Table I are averages of the results of two independent observations. Measurements on λ3755 and λ3710 yield similar results.

TABLE I

VARIATION WITH EXCITING VOLTAGE OF INTENSITY RATIOS ON THE CONTOUR OF λ3805

Accelerating potential, volts	I_a/I_m	I_b/I_m	I_b/I_a
14.0	0.135	0.082	0.61
15.0	0.105	0.058	0.55
16.0	0.098	0.046	0.46
18.0	0.085	0.037	0.44

The intensity-blackening calibration curves were established over the range of intensities given here, by putting two sets of calibration marks on the plate. By no stretch of the imagination can they be so distorted that they give constant intensity ratios for I_a/I_m, I_b/I_m and I_b/I_a.

The values in Table II are the averages of the temperatures found from the two independent sets of data, and are followed by the deviation of the individual values from the mean.

TABLE II

DISTRIBUTION TEMPERATURES FOUND FROM THE OBSERVED INTENSITY RATIOS, BY ASSUMING A BOLTZMANN DISTRIBUTION IN THE INITIAL LEVELS

Accelerating potential, volts	T_1 ?κ $\left(\frac{I_a}{I_m}\right)$	T_2 ?κ $\left(\frac{I_b}{I_m}\right)$	T_3 ?κ $\left(\frac{I_b}{I_a}\right)$
14.0	770 ± 50	890 ± 15	1280 ± 0
15.0	650 ± 20	760 ± 10	1030 ± 180
16.0	620	690	750
18.0	570 ± 50	620 ± 20	710 ± 70

Discussion

Table I shows that the intensity of λ3805 near the tail is considerably greater, relative to the maximum intensity, at 14 volts than it is at 18 volts accelerating potential. Moreover there is a progressive change as the voltage is varied between these limits. This can result only from a relative decrease in the populations of the higher rotational levels with increase in exciting voltage. We shall first consider the results of an approximate analysis of the contours, and later the probable causes of the observed behavior.

If the initial rotational level populations are in a Boltzmann distribution, the effective temperatures determined from any three points on a contour should agree (see section on "Contour analysis"). The results in Table II indicate a higher temperature the farther the considered points are from the maximum. At 18 volts the differences are small and may possibly not be significant, but at 14 volts there are very definite differences. The intensity ratios predicted for the Boltzmann distributions which fit one intensity ratio at each voltage, differ from the observed values for the other two by as much as 35%. In addition to this effect, the calculated temperatures increase as the accelerating potential is lowered.

It is not possible to represent the results by assuming two superimposed Boltzmann distributions. All intensity ratios, however, are given to better than 4% by a composite distribution having a temperature θ from $\kappa = 0$ to $\kappa = 20$, and a temperature T_3 (Table II) for the higher levels. Since I_m is predominantly influenced by the populations of low levels, and I_a and I_b by the levels $\kappa = 20$ to 26, and $\kappa = 25$ to 30, it is probably a rough approximation to the actual distribution. The values of θ at 18, 16, 15 and 14 volts are 550, 570, 560 and 600° K. These will be referred to later. The calculated populations of the $\kappa = 9$, 20, 30 and 40 individual levels relative to that of the $\kappa = 0$ level are 3, 7, 150 and 700% greater at 14 volts than they are at 18 volts.

The individual level populations may be expressed in terms of the number of excited molecules, M, in the $v' = 0$ vibration state by treating κ as a variable and integrating Equation (2) from 0 to 19 with a temperature θ, and from 20 to infinity with a temperature T_3. The factor which makes the two parts of the distribution equal at $\kappa = 20$ must be taken into account. The relative value of M at each voltage is known from the total intensity of the vibration band (4, 9). Hence it is possible to compare the populations of any given level at different voltages. For example the $\kappa = 40$ level would have a 50% greater population at 14 volts than it has at 18 volts, although the ratio of the M values is 0.25.

The present results differ in character from those of other experimenters, who used similar excitation tubes but higher exciting voltages. The latter indicate a Boltzmann distribution in the initial levels (7, 8). Moreover it is probable that the distribution temperature is determined by the temperature of the cage walls. Ornstein and Van Wijk, and Van Wijk (10, 12; 13, p. 586), have shown by direct measurement that this is so when the molecules are excited inside a hollow cylinder. Lindh's results indicate an increase of 30 or 40° C. in the distribution temperature for an exciting voltage increase from 150 to 175 volts. If his single intensity measurements were made to within 8%, the greatest accuracy obtainable in the average distribution temperatures was 3% (a possible error of 17° C.). The average values for the relative intensities for each set of conditions varied from the corresponding values for the other sets by less than 6%, with one exception. On the other hand, the two sets of values for the relative intensities for each particular set of conditions showed variations of as much as 8%. One must accept the results, however, as showing some increase in the distribution temperature with increase in exciting voltage. The writer thinks that this is probably due, not to a direct dependence of the rotational distribution on the exciting voltage, but rather to an increase in the cage wall temperature (*cf.* reference 1). The cage temperature is determined by the equilibrium condition between heat gained by electron bombardment, gaseous conduction, and absorption of radiation from the filament, and heat lost by conduction, mainly through the single supporting wire. Owing to the high accelerating potentials used by Lindh, the second and third factors remained practically constant for constant tube current, so that an increase in cage temperature is expected because of the increased energy input. The increase, estimated from the known construction characteristics of the tube, is of the right order of magnitude. It may be that the temperature decrease expected for a decrease in tube current is not apparent because of the rather wide limits of accuracy of the temperature measurements. The experiments of Ornstein and Kruithof on hydrogen (8) furnish additional supporting evidence. They found the distribution temperature independent of the exciting voltage between 20 and 30 volts, but dependent on tube current, *i.e.*, filament temperature. This can be understood from the increased cage temperature (due to increased radiation and gaseous conduction) with increase in filament temperature. When the exciting voltage is decreased under these conditions, the filament temperature must be materially increased and so tends to compensate for the lowered energy input. The fact that these authors found lower distribution temperatures (about 300° *K*) than those found by Lindh (about 560° *K*) may result from the lower gas pressure (0.03 rather than 0.1 mm. of mercury) and consequently lower gaseous conduction, which under these conditions depends on the pressure. (In this type of tube the filament is placed as close as possible to the cage grid.)

In contrast to these results the characteristic feature of the present results was a favoring of the populations of high levels at 14 volts, which decreased as the voltage increased, so that the distribution tended to become a Boltz-

mann distribution at about 18 volts. In addition, the higher the level the more it appeared to be favored. The explanation must be sought either in some temperature effect due to the higher filament temperatures necessary for constant tube current at the lower voltages, or in a direct dependence of the relative populations of the initial rotational levels on the energy of the exciting electrons.

An estimate of the increase in the temperature of the cage and the filament when the exciting voltage dropped from 18 to 14 volts was obtained from a duplicate tube. The former was measured with a thermocouple and the latter with an optical pyrometer. Various adjustments of filament, guard ring and cage were made to overcome slight divergences of the duplicate from the original. The maximum filament temperature increase was 200° C. and the corresponding cage temperature increase was 26° C. The latter was probably somewhat higher in the original tube since the pressure was somewhat greater.

The solid angle subtended by the filament at the point from which the observed light came was certainly less than $\frac{1}{100}$ of that subtended by the cage walls. Since the accommodation coefficient for nitrogen is comparable to 1 (13), it is to be expected that the only effect of the rise in filament temperature was to increase the temperature of the cage walls. This view is supported by the work of Ornstein and Kruithof, in which a current variation between 0.2 and 1.5 ma. at constant voltage did not destroy the Boltzmann distribution, but increased the distribution temperature. The filament temperature changes involved were certainly greater than those in the present work. The observed effects might be explained by a remarkable persistence of rotation on collision with a wall for rapidly, but not for slowly, rotating molecules, but this should occur as well in other experiments with similar tubes, and a Boltzmann distribution has consistently been found.

On the other hand, energy interchange between the impinging electron and the molecular rotation is expected to be probable only when the two energies are comparable. Since the rotational energy of the molecule in the $\varkappa = 20$ level of the ground state is only 0.1 electron volt, this is unlikely to be the explanation. The number and character of the slow secondary electrons in the tube probably do not change rapidly enough with the exciting voltage to account for the observed behavior on the basis of their interaction with the molecular rotation.

The observed results as well as their relation to those of other investigators could be understood, however, if there is an appreciable probability of a double switch between the energy of the exciting electron and the molecular electronic and rotational energy. One can think that an electron after exciting the electronic configuration, has a definite probability of interacting with the rotation before escaping from the molecular field. The energy relations are such as to make this possible. The exciting voltage at which the bands first appear for this set-up is easily obtained from extrapolation of the optical excitation function (4, 9). The value so obtained is 13.4 volts. A 14 volt electron then has an energy of 0.6 electron volt after exciting the electronic

configuration. This is of the order of the rotational energy (0.28 electron volt for $\kappa = 35$), and might be expected to interact with it. As the energy of the exciting electrons is increased the excess energy becomes greater and the probability of interaction consequently less, until a point is reached at which it is improbable. Above this point one would expect to find a Boltzmann distribution.

If this explanation is correct the populations of the low levels should be in an almost Boltzmann distribution with a temperature determined by the cage temperature. In the composite distribution which gives the correct intensity ratios, the low level distribution temperatures, θ, varied from 550° K at 18 volts to 600° K at 14 volts. The increase is of the magnitude of the increase in cage temperature found by direct measurement in the duplicate tube. Moreover the distribution temperatures are about the same as those found by Lindh for the negative band λ3914, with higher exciting voltages but otherwise identical conditions. It is known that λ3914 and λ3805 have the same rotational distribution temperature in the same discharge (12). The θ values seem to be reasonable therefore, in view of the compensating effects of increased filament current and decreased energy input.

Although the evidence for this explanation cannot be regarded as conclusive because of the approximate nature of the analysis, there are many points in its favor. The fact that the departure from a Boltzmann distribution is great at 14 volts and slight (if any) at 18 volts is especially encouraging. Determination of the validity of the explanation must rest on further measurements of the intensities of individual rotation lines.

In conclusion it should be pointed out that all remarks made in this article, especially those on page 11, refer *only* to the excitation of molecules by electronic impact. When there are also collisions between molecules and heavy particles moving under the influence of an electric field, the problem becomes extremely complicated, and the distribution temperature in the excited states may be expected to be related in no simple way to the actual gas temperature [*Cf.* reference 11].

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STANDARD NOMOGRAPHIC FORMS FOR EQUATIONS IN THREE VARIABLES[1]

By F. M. Wood[2]

Abstract

Equations of the third and fourth nomographic order in three variables have been dealt with and classified. Equations of the third order may be reduced to one of two standard forms, $\alpha + \beta + \gamma = 0$ and $\alpha + \beta\gamma = 0$, which give alignment charts composed of three straight lines. Equations of the fourth order may also be reduced to one of two standard forms, resulting in charts composed of (*a*) two straight lines and a curve, or (*b*) two scales on a conic, and the third on another curve. Transformations of these four standard forms are given which permit of rapid and easy adjustment of the position and length of the scales for any given example, resulting in a chart of practical utility. Although the underlying theory has been studied by other writers, notably Soreau and Clark, it has possibly never appeared before in such a neat form. On this account, and also because of the standard transformations, it is felt that this article is of particular value.

Standard forms have also been developed for third order equations leading to charts composed of two scales on a conic and a third straight scale, and in conclusion a third type of chart, in which all three scales appear on a single cubic curve, has been standardized. The practical value of the last type is questionable, but the conic charts are of use since we may arbitrarily choose the unit circle, or the rectangular hyperbola, for our conic scales. Final adjustment forms which permit suitable location of the scales in particular examples have been obtained in every case.

Introduction

In this article an attempt is made to standardize for practical use the work which has been done on the representation by alignment charts of equations in three variables. Of particular note in this respect is the work of R. Soreau (5–8) and of J. Clark (1–4). Although the fundamental ideas given here are not original, it is hoped that the method of presentation and the arrangement will carry an appeal, especially to those having occasion from time to time to construct such charts.

The equation

$$f_3(a_0f_1f_2 + a_1f_1 + a_2f_2 + a_3) + \phi_3(b_0f_1f_2 + b_1f_1 + b_2f_2 + b_3) + (c_0f_1f_2 + c_1f_1 + c_2f_2 + c_3) = 0$$

is said to be the general equation of the fourth order. Here f_1, f_2 and f_3 are functions of three independent variables, and ϕ_3 is a function of the same variable as f_3, such that it cannot be expressed as a linear form of f_3, *i.e.*, we cannot express ϕ_3 in the form $d_1f_3+d_2$, where d_1 and d_2 are constants. Also a_0, etc., b_0, etc., and c_0, etc., are constants.

The general third order equation may be written

$$f_3(a_0f_1f_2 + a_1f_1 + a_2f_2 + a_3) + (b_0f_1f_2 + b_1f_1 + b_2f_2 + b_3) = 0.$$

This is a particular case of the third order equation when $\phi_3 = 0$, or can be expressed as a linear form of f_3.

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It has been proved that any third order equation may be represented by an alignment chart composed of three straight line scales, one for each of the variables. In most cases a simple algebraic transformation leads to such a chart, whereas in certain special cases a transcendental transformation employing inverse tangents is required. It has also been proved that any third order equation may be represented by a chart in which one of the variables (any one of the three) has a straight line scale, and the other two have scales located on a conic. The value of this type of chart is apparent when it is stated that a circle may be chosen for this conic, and that the two scales may be shifted on the conic independently of each other to suitable positions. Thirdly, it has been proved that any third order equation may be represented by a chart in which all three scales are located on a single curve, which is a cubic, and that in this case also the three scales may be shifted independently of one another along the cubic.

For fourth order equations it has been proved that two types of chart, mutually exclusive, are possible. In one type two of the scales lie on straight lines (f_1 and f_2) and the third scale ($f_3\phi_3$) lies on a curve. In the other type the two scales lie on a conic and the third scale as before lies on another curve. In the latter type the conic scales may be shifted along the conic, but not independently of each other. Although this does not give the freedom of adjustment present in the third order charts, it nevertheless permits suitable placing of the third scale with respect to the conic.

Soreau has studied the theory for the straight line representation of third order equations, and Clark has studied the fourth order equation, and the conic and cubic representation of the third order equation. Clark developed a neat geometric (synthetic) method of constructing the charts, using the anharmonic properties of rays intersecting on a conic.

In the following analysis, the general equations are subdivided into various types, and a standard form is found for each type, and standard transformations of these forms are developed to give suitable charts for any particular example,—charts in which the three scales, covering the particular range of values of the three variables, will be advantageously placed with respect to one another. The analytical method, with rectangular co-ordinates, is used in preference to the geometric construction of Clark, as it is felt that the latter method is not amenable to standardization, not so simple to study and discuss, and, under certain conditions which may arise in any example, is not so accurate.

Alignment Charts of Three Straight Lines for Third Order Equations

The general third order equation is

$$f_3(a_0f_1f_2 + a_1f_1 + a_2f_2 + a_3) + (b_0f_1f_2 + b_1f_1 + b_2f_2 + b_3) = 0. \qquad (1)$$

It will be shown that this may be reduced to one or other of the two canonical forms $\alpha+\beta+\gamma=0$ — (A), $\alpha+\beta\gamma=0$ — (B); and that both of these forms result in charts composed of three straight lines. These canonical forms are then adjusted to give suitable position for the scales in particular examples.

It is assumed that the constant $a_0 \neq 0$, so that the term containing $f_1 f_2 f_3$ is present. If such is not the case in any given equation, a division by f_1, f_2, or $f_1 f_2$ will put it in the desired form. It will be found that division throughout by a_0, making the coefficient of $f_1 f_2 f_3$ unity, simplifies the work considerably.

Writing $X_1 = f_1 + \frac{a_2}{a_0}$; $X_2 = f_2 + \frac{a_1}{a_0}$; $X_3 = f_3 + \frac{b_0}{a_0}$ we reduce to

$$X_1X_2X_3 + B_1X_1 + B_2X_2 + B_3X_3 + 2P = 0 \ , \tag{2}$$

where $B_1 = \frac{1}{a_0^2}(a_0b_1 - a_1b_0)$; $B_2 = \frac{1}{a_0^2}(a_0b_2 - a_2b_0)$; $B_3 = \frac{1}{a_0^2}(a_0a_3 - a_1a_2)$;

$$2P = \frac{1}{a_0^2}\left[(a_0b_3 - a_3b_0) - a_0a_2B_1 - a_0a_1B_2\right].$$

Now substitute $B_1X_1 = a\phi_1 + b$; $B_2X_2 = a\phi_2 + b$; $B_3X_3 = a\phi_3 + b$.
The new form

$$A_0\phi_1\phi_2\phi_3 + A_1(\phi_1\phi_2 + \phi_1\phi_3 + \phi_2\phi_3) + A_2(\phi_1 + \phi_2 + \phi_3) + A_3 = 0 \ , \tag{3}$$

results, where $A_0 = a^3$; $A_1 = a^2b$; $A_2 = ab^2 + aB_1B_2B_3$; $A_3 = b^3 + B_1B_2B_3(3b + 2P)$.
(*a*) If in this symmetrical form we apply the conditions $A_0 = A_2$; $A_1 = A_3$ we determine particular values of the constants a and b to satisfy, *e.g.*, $a = \sqrt{P^2 + B_1B_2B_3} = R$; $b = -P$.

Equation (3) then reduces to

$$(\phi_1\phi_2\phi_3 + \Sigma\phi_1)R - (\Sigma\phi_1\phi_2 + 1)P = 0 \quad \text{or} \quad \frac{\phi_1\phi_2\phi_3 + \Sigma\phi_1}{\Sigma\phi_1\phi_2 + 1} = \frac{P}{R} \ .$$

Hence $\frac{\phi_1\phi_2\phi_3 + \Sigma\phi_1\phi_2 + \Sigma\phi_1 + 1}{\phi_1\phi_2\phi_3 - \Sigma\phi_1\phi_2 + \Sigma\phi_1 - 1} = \frac{P+R}{P-R}$; or $\frac{\phi_1+1}{\phi_1-1} \cdot \frac{\phi_2+1}{\phi_2-1} \cdot \frac{\phi_3+1}{\phi_3-1} = \frac{P+R}{P-R}$.

Re-substituting for ϕ_1, ϕ_2, ϕ_3 this becomes

$$\frac{B_1X_1 + P + R}{B_1X_1 + P - R} \cdot \frac{B_2X_2 + P + R}{B_2X_2 + P - R} \cdot \frac{B_3X_3 + P + R}{B_3X_3 + P - R} = \frac{P+R}{P-R} \ , \tag{4}$$

which can be put in the canonical form $\alpha + \beta\gamma = 0$ — (*B*) if we write

$$\alpha = \frac{P+R}{P-R} \cdot \frac{B_1X_1 + P - R}{B_1X_1 + P + R} \ ; \ \beta = \frac{B_2X_2 + P + R}{B_2X_2 + P - R} \ ; \ \gamma = -\frac{B_3X_3 + P + R}{B_3X_3 + P - R} \ .$$

The symmetry of Equation (4) permits a choice of the functions α, β, and γ. However, it may be used only when $P^2 + B_1B_2B_3 > 0$.

(*b*) When $P^2 + B_1B_2B_3 < 0$, the same analysis as for Case (*a*) may be used as far as Equation (3). At this point, the conditions applied are $A_0 = -A_2$; $A_1 = -A_3$; and the resulting values of a and b are $a = \sqrt{-P^2 - B_1B_2B_3} = R_1$; $b = -P$.

Hence we may obtain $(\phi_1\phi_2\phi_3 - \Sigma\phi_1)R_1 - (\Sigma\phi_1\phi_2 - 1)P = 0$, which results in

$$\frac{\phi_1 + \phi_2 + \phi_3 - \phi_1\phi_2\phi_3}{1 - \phi_1\phi_2 - \phi_2\phi_3 - \phi_3\phi_1} = \frac{P}{R_1} \ .$$

If this is compared with the expansion of $\tan(A+B+C)$ in terms of $\tan A$, $\tan B$, and $\tan C$, it is evident that the equation may be written

$$\tan^{-1}\phi_1 + \tan^{-1}\phi_2 + \tan^{-1}\phi_3 = \tan^{-1}\frac{P}{R_1} \ ,$$

i.e.,

$$\tan^{-1}\frac{B_1X_1 + P}{R_1} + \tan^{-1}\frac{B_2X_2 + P}{R_1} + \tan^{-1}\frac{B_3X_3 + P}{R_1} = \tan^{-1}\frac{P}{R_1} \ .$$

Here $\tan^{-1}\frac{P}{R_1}$ may be combined with one of the other three to give

$$\tan^{-1}\frac{B_1X_1+P}{R_1}+\tan^{-1}\frac{B_2X_2+P}{R_1}+\tan^{-1}\frac{R_1X_3}{PX_3-B_1B_2}=0\ , \tag{5}$$

which is evidently of the canonical form $\alpha+\beta+\gamma=0$ — (A). This may be used only when $P^2+B_1B_2B_3<0$. The scales for this chart are periodic, being graduated according to the angular measures of $\tan^{-1}\frac{B_1X_1+P}{R_1}$, etc., which have a period of 180°. However, this need not lead to confusion in plotting, since the two outer scales may be chosen and adjusted for position first, using preferably the period from 0° to 180°. The third scale (*i.e.*, some period of it) will of necessity fall in between these two outer scales.

(*c*) When $P^2+B_1B_2B_3=0$, and all these coefficients B_1, B_2, B_3, P, differ from zero.

Substitute $\phi_1=\frac{B_1X_1}{P}$; $\phi_2=\frac{B_2X_2}{P}$; $\phi_3=\frac{B_3X_3}{P}$ and Equation (2) reduces to

$$\phi_1\phi_2\phi_3-(\phi_1+\phi_2+\phi_3)-2=0.$$

Substituting further $\phi_1=F_1-1$, and similarly for ϕ_2, ϕ_3, we obtain

$$F_1F_2F_3-(F_1F_2+F_2F_3+F_3F_1)=0.$$

This, on division throughout by $F_1F_2F_3$ and re-substitution, gives

$$\frac{P}{B_1X_1+P}+\frac{P}{B_2X_2+P}+\frac{P}{B_3X_3+P}=1\ , \tag{6}$$

which is of the canonical form $\alpha+\beta+\gamma=0$, if we combine one of the three functions with unity on the right-hand side.

The above three cases are applicable when all of B_1, B_2, B_3, P, differ from zero. Following are the special cases when one or more of these are zero.

(*d*) $X_1X_2X_3+B_2X_2+B_3X_3+2P=0$ (one of the coefficients B_1, B_2, B_3, zero).

Dividing through by X_2X_3, and substituting

$$\frac{B_2}{X_3}=\phi_3-\frac{B_2B_3}{2P}\ ;\ \frac{B_3}{X_2}=\phi_2-\frac{B_2B_3}{2P}\ ;$$

this reduces to

$$\left(X_1-\frac{B_2B_3}{2P}\right)+\frac{2P}{B_2B_3}\cdot\phi_2\phi_3=0 \quad \text{or} \quad \frac{1}{2P}\left(X_1-\frac{B_2B_3}{2P}\right)+\left(\frac{1}{X_2}+\frac{B_2}{2P}\right)\left(\frac{1}{X_3}+\frac{B_3}{2P}\right)=0$$

which may be still further reduced to

$$(2PX_1-B_2B_3)+\left(\frac{2P}{X_2}+B_2\right)\left(\frac{2P}{X_3}+B_3\right)=0 \tag{7}$$

of the canonical form $\alpha+\beta\gamma=0$.

(*e*) $X_1X_2X_3+B_2X_2+B_3X_3=0$ ($P=0$ and one of B_1, B_2, B_3 zero).

Dividing through by X_2X_3 reduces this immediately to

$$X_1+\frac{B_2}{X_3}+\frac{B_3}{X_2}=0 \tag{8}$$

of the canonical form $\alpha+\beta+\gamma=0$.

(*f*) $X_1X_2X_3+B_3X_3+2P=0$ (Two of B_1, B_2, B_3, zero).

Dividing through by X_3, this gives

$$\left(\frac{2P}{X_3}+B_3\right)+X_1X_2=0 \tag{9}$$

of the canonical form $\alpha+\beta\gamma=0$.

(g) $X_1X_2X_3+2P=0$ (All three of B_1, B_2, B_3, zero).

Dividing through by X_3, this gives

$$\frac{2P}{X_3}+X_1X_2=0 \tag{10}$$

of the canonical form $\alpha+\beta\gamma=0$.

The above analysis includes all cases of the third order equation. Any particular example for which an alignment chart is required may be reduced to one of these seven cases, and expressions for α, β, and γ determined in terms of the original three functions f_1, f_2, and f_3. Adjustments of the α, β, and γ scales on the charts to suit particular ranges of value of the three variables will now be studied.

Type I. $\alpha+\beta+\gamma=0$, with three parallel straight scales.

The fundamental determinant form is

$$\begin{vmatrix} 0 & \alpha & 1 \\ 1 & -\frac{1}{2}\beta & 1 \\ 2 & \gamma & 1 \end{vmatrix} = 0 \ .$$

Here the α scale lies on the straight line $x=0$, graduated $y=\alpha$;

The β scale lies on the straight line $x=1$, graduated $y=-\frac{1}{2}\beta$;

The γ scale lies on the straight line $x=2$, graduated $y=\gamma$.

These scales should be shifted so that they lie opposite one another. To do this we use the transformation

$$\begin{vmatrix} 0 & \alpha & 1 \\ \frac{2}{m+1} & \frac{-m\beta+n}{m+1} & 1 \\ 2 & m\gamma+n & 1 \end{vmatrix} = 0$$

where we have magnified the γ scale m times with respect to the α scale, and then shifted it up a distance n. If the desired chart is to be rectangular in shape, and the extreme values of the variables α and γ are denoted by the subscripts 1 and 2, we must have $\alpha_1=m\gamma_1+n$; $\alpha_2=m\gamma_2+n$. Solving for m and n, this will give the determinant form which places the α and γ scales opposite each other. The β scale must necessarily be placed suitably between these two.

Should it be desired to magnify the β scale instead of the γ scale, the transformation to use is

$$\begin{vmatrix} 0 & \alpha & 1 \\ 1 & -\frac{m\beta}{2}+n & 1 \\ \frac{2}{2-m} & \frac{m\gamma+2n}{2-m} & 1 \end{vmatrix} = 0 \ .$$

Owing to symmetry, any two of the three variables may be chosen for the outside scales.

Type II. $\alpha+\beta+\gamma=0$, with three concurrent straight scales.

The fundamental determinant form here is

$$\begin{vmatrix} -\frac{1}{\alpha} & -\frac{1}{\alpha} & 1 \\ 0 & \frac{1}{\beta} & 1 \\ \frac{1}{\gamma} & 0 & 1 \end{vmatrix} = 0 ,$$

and the transformation

$$\begin{vmatrix} \frac{1+k}{m+n-\alpha} & \frac{1}{m+n-\alpha} & 1 \\ \frac{k}{\beta+m} & \frac{1}{\beta+m} & 1 \\ \frac{1}{\gamma+n} & 0 & 1 \end{vmatrix} = 0 .$$

In studying this transformation it is seen that the γ scale lies along the x axis, and the other two scales meet it at the origin. Trial alone will indicate in any example the best values to assign to k, m, and n. The use of k frees us from the necessity of using oblique axes; m and n ensure that the scales do not extend to infinity, and also permit adjustment so that the most important parts of the scales, where greatest accuracy is required, shall lie furthest from the origin.

Type III. $\alpha+\beta\gamma=0$, with two parallel and one inclined straight scale.

The six forms listed below cover this type. If, however, the equation is expressed $-\beta \cdot \gamma \cdot \frac{1}{\alpha}=1$, or its reciprocal $-\frac{1}{\beta}\cdot\frac{1}{\gamma}\cdot\alpha=1$, it is seen that a choice is possible in assigning α, β, and γ to the three variables, and when this is done only the first of the six forms is necessary.

$$(a)\ \begin{vmatrix} 0 & \beta & 1 \\ 1 & \alpha & 1 \\ \frac{1}{1+\gamma} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & m\beta+n & 1 \\ 1 & \alpha & 1 \\ \frac{m}{m+\gamma} & \frac{n\gamma}{m+\gamma} & 1 \end{vmatrix} \qquad (b)\ \begin{vmatrix} 0 & \beta & 1 \\ 1 & -\alpha & 1 \\ \frac{1}{1-\gamma} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & m\beta+n & 1 \\ 1 & -\alpha & 1 \\ \frac{m}{m-\gamma} & \frac{-n\gamma}{m-\gamma} & 1 \end{vmatrix}$$

$$(c)\ \begin{vmatrix} 0 & \frac{1}{\alpha} & 1 \\ 1 & \frac{1}{\beta} & 1 \\ \frac{1}{1+\gamma} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & \frac{m}{\alpha}+n & 1 \\ 1 & \frac{1}{\beta} & 1 \\ \frac{m}{m+\gamma} & \frac{n\gamma}{m+\gamma} & 1 \end{vmatrix} \qquad (d)\ \begin{vmatrix} 0 & \frac{1}{\alpha} & 1 \\ 1 & -\frac{1}{\beta} & 1 \\ \frac{1}{1-\gamma} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & \frac{m}{\alpha}+n & 1 \\ 1 & -\frac{1}{\beta} & 1 \\ \frac{m}{m-\gamma} & \frac{-n\gamma}{m-\gamma} & 1 \end{vmatrix}$$

$$(e)\ \begin{vmatrix} 0 & \frac{1}{\gamma} & 1 \\ 1 & \beta & 1 \\ \frac{1}{1+\alpha} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & \frac{m}{\gamma}+n & 1 \\ 1 & \beta & 1 \\ \frac{m}{m+\alpha} & \frac{n\alpha}{m+\alpha} & 1 \end{vmatrix} ; \quad (f)\ \begin{vmatrix} 0 & \frac{1}{\gamma} & 1 \\ 1 & -\beta & 1 \\ \frac{1}{1-\alpha} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & \frac{m}{\gamma}+n & 1 \\ 1 & -\beta & 1 \\ \frac{m}{m-\alpha} & \frac{-n\alpha}{m-\alpha} & 1 \end{vmatrix}$$

Type IV. $\alpha+\beta\gamma=0$, with two intersecting scales and a third straight scale.

The fundamental determinant here is

$$\begin{vmatrix} 0 & \frac{1}{\alpha+n} & 1 \\ \frac{\beta}{\beta+n} & \frac{1}{\beta+n} & 1 \\ \frac{1}{1+\gamma} & 0 & 1 \end{vmatrix} = 0 \ .$$

This form can replace Type III (*c*) when either or both of α and β pass through zero. The transformation here may be written

$$\begin{vmatrix} 0 & \frac{1}{\alpha+n} & 1 \\ \frac{\beta}{m\beta+n} & \frac{k\beta+1}{m\beta+n} & 1 \\ \frac{1}{m+\gamma} & \frac{k}{m+\gamma} & 1 \end{vmatrix} = 0 \ ;$$

where k removes any necessity to employ oblique axes, and m and n allow adjustment of the position of the γ and α scales with respect to each other, and also the origin.

Fourth Order Equations

The general form is

$$f_3(a_0f_1f_2+a_1f_1+a_2f_2+a_3)+\phi_3(b_0f_1f_2+b_1f_1+b_2f_2+b_3) + (c_0f_1f_2+c_1f_1+c_2f_2+c_3)=0 \ , \tag{11}$$

where f_3 and ϕ_3 are not connected by a linear relation $\phi_3=d_1f_3+d_2$ (otherwise this general equation reduces to the third order). We may write the general equation

$$f_1f_2A_3+f_1B_3+f_2C_3+D_3=0 \tag{12}$$

where

$$A_3=a_0f_3+b_0\phi_3+c_0 \ ; \qquad B_3=a_1f_3+b_1\phi_3+c_1 \ ;$$
$$C_3=a_2f_3+b_2\phi_3+c_2 \ ; \qquad D_3=a_3f_3+b_3\phi_3+c_3 \ .$$

(*i*) When one of the expressions A_3, B_3, C_3, D_3 is identically zero.

(*a*) For $A_3=0$, the general form reduces to $\frac{D_3}{B_3}+f_1+\frac{C_3}{B_3}f_2=0$ (13)

(*b*) For $B_3=0$, the general form reduces to $\frac{C_3}{A_3}+f_1+\frac{D_3}{A_3}\cdot\left(\frac{1}{f_2}\right)=0$ (14)

(*c*) For $C_3=0$, the general form reduces to $\frac{B_3}{A_3}+f_2+\frac{D_3}{A_3}\left(\frac{1}{f_1}\right)=0$ (15)

(*d*) For $D_3=0$, the general form reduces to $\frac{A_3}{C_3}+\frac{1}{f_1}+\frac{B_3}{C_3}\left(\frac{1}{f_2}\right)=0$. (16)

(Should two of these expressions be zero, the case reduces to the third order).

Each of the above equations is of the canonical form $\alpha_1+\beta+\alpha_2\gamma=0$—(*C*), which may be represented by a chart with two straight scales for the variables β and γ, and one curved scale for the variable $[\alpha_1\alpha_2]$, which is in each case the original variable $[f_3\phi_3]$. The transformations for this canonical form will be discussed at the conclusion of Case (*ii*) below.

When none of A_3, B_3, C_3, D_3 are zero, it is necessary to evaluate the four quantities

$$a=\begin{vmatrix} a_1 & b_1 & c_1 \\ a_2 & b_2 & c_2 \\ a_3 & b_3 & c_3 \end{vmatrix};\ b=\begin{vmatrix} a_0 & b_0 & c_0 \\ a_2 & b_2 & c_2 \\ a_3 & b_3 & c_3 \end{vmatrix};\ c=\begin{vmatrix} a_0 & b_0 & c_0 \\ a_1 & b_1 & c_1 \\ a_3 & b_3 & c_3 \end{vmatrix};\ d=\begin{vmatrix} a_0 & b_0 & c_0 \\ a_1 & b_1 & c_1 \\ a_2 & b_2 & c_2 \end{vmatrix}.$$

It will be shown that if $ad=bc$, a chart is possible composed of two straight scales for f_1 and f_2, and a curved scale for $[f_3\phi_3]$, but that if $ad\neq bc$ such a chart is impossible, the scales for f_1 and f_2 must be placed on a conic and the scale for $[f_3\phi_3]$ on a curve.

When none of A_3, B_3, C_3, D_3 are zero, it may be proved that one at least of a, b, c, d, differs from zero. It can also be shown that the identity

$$aA_3 - bB_3 + cC_3 - dD_3 = 0 \tag{17}$$

is true. Thus no three of a, b, c, d may be zero.

Suppose $d\neq 0$. Then eliminating D_3 between Equations (12) and (17) we obtain $\left(f_1f_2+\frac{a}{d}\right)A_3+\left(f_1-\frac{b}{d}\right)B_3+\left(f_2+\frac{c}{d}\right)C_3=0$.

Substitute $X_1=f_1-\frac{b}{d}$; $X_2=f_2+\frac{c}{d}$, with the result

$$X_1X_2+X_1\left(\frac{B_3}{A_3}-\frac{c}{d}\right)+X_2\left(\frac{C_3}{A_3}+\frac{b}{d}\right)+\frac{ad-bc}{d^2}=0\ . \tag{18}$$

(*ii*) For $ad=bc$ the independent term in Equation (18) vanishes. Substitute back for X_1 and X_2 and divide through by $X_1X_2\left(\frac{C_3}{A_3}+\frac{b}{d}\right)$ and obtain

$$\frac{A_3}{dC_3+bA_3}+\frac{1}{df_1-b}+\frac{dB_3-cA_3}{dC_3+bA_3}\cdot\frac{1}{df_2+c}=0\ . \tag{19d}$$

In a similar manner, by assuming each in turn of a, b, c, differing from zero, three other similar forms are obtained. In any example there is a choice of two of the four forms. The three remaining forms follow:—

For $a\neq 0$, $\frac{D_3}{aB_3+cD_3}+\frac{f_1}{a-cf_1}+\frac{aC_3-bD_3}{aB_3+cD_3}\cdot\frac{f_2}{a+bf_2}=0$ (19a)

For $b\neq 0$, $\frac{C_3}{bA_3+dC_3}+\frac{f_1}{b-df_1}+\frac{bD_3-aC_3}{bA_3+dC_3}\cdot\frac{1}{a+bf_2}=0$ (19b)

For $c\neq 0$, $\frac{B_3}{aB_3+cD_3}+\frac{1}{cf_1-a}+\frac{cA_3-dB_3}{aB_3+cD_3}\cdot\frac{f_2}{c+df_2}=0$ (19c)

The above four Equations (19) are of the type $\alpha_1+\beta+\alpha_2\gamma=0$ — (*C*) which will now be discussed.

Type V. $\alpha_1+\beta+\alpha_2\gamma=0$, with two parallel and one curved scale.

The fundamental determinants are

$$\begin{vmatrix} \frac{1}{1+\alpha_3} & \frac{-\alpha_1}{1+\alpha_3} & 1 \\ 0 & \gamma & 1 \\ 1 & \beta & 1 \end{vmatrix}=0\ .$$

and

$$\begin{vmatrix} \frac{1}{1-\alpha_3} & \frac{\alpha_1}{1-\alpha_3} & 1 \\ 0 & \gamma & 1 \\ 1 & -\beta & 1 \end{vmatrix}=0\ .$$

Note that the β scale has been reversed with respect to the γ scale.

These give rise to the transformations

$$\begin{vmatrix} \frac{1}{1+m\alpha_2} & \frac{n-m\alpha_1}{1+m\alpha_2} & 1 \\ 0 & \gamma & 1 \\ 1 & m\beta+n & 1 \end{vmatrix} = 0 \text{ and } \begin{vmatrix} \frac{1}{1-m\alpha_2} & \frac{n+m\alpha_1}{1-m\alpha_2} & 1 \\ 0 & \gamma & 1 \\ 1 & n-m\beta & 1 \end{vmatrix} = 0 .$$

The constant m magnifies and the constant n translates the β scale.

Type VI. $\alpha_1+\beta+\alpha_2\gamma=0$, with two intersecting straight scales and a curved scale.

The fundamental and transformed determinants here are

$$\begin{vmatrix} -\frac{\alpha_2}{\alpha_1} & -\frac{1}{\alpha_1} & 1 \\ 0 & \frac{1}{\beta} & 1 \\ \frac{1}{\gamma} & 0 & 1 \end{vmatrix} = 0 \text{ and } \begin{vmatrix} \frac{\alpha_2+k}{m+n\alpha_2-\alpha_1} & \frac{1}{m+n\alpha_2-\alpha_1} & 1 \\ \frac{k}{\beta+m} & \frac{1}{\beta+m} & 1 \\ \frac{1}{\gamma+n} & 0 & 1 \end{vmatrix} = 0 .$$

Remarks similar to those for Type II above apply here.

(*iii*) Fourth order equations requiring conic scales.

When $ad \neq bc$, the independent term of Equation (18) does not vanish. Writing $k=\frac{ad-bc}{d^2}$; $F_3=\frac{B_3}{A_3}-\frac{c}{d}$; $G_3=\frac{C_3}{A_3}+\frac{b}{d}$ and dividing through by X_2 this equation becomes

$$\left(X_1\frac{k}{X_2}\right)\frac{F_3}{K}+\left(X_1+\frac{k}{X_2}\right)+G_3=0 . \tag{20}$$

This is symmetric in the functions X_1 and $\frac{k}{X_2}$ and may be expressed

$$\begin{vmatrix} -1 & G_3 & \frac{F_3}{k} \\ X_1 & X_1^2 & 1 \\ \frac{k}{X_2} & \left(\frac{k}{X_2}\right)^2 & 1 \end{vmatrix} = 0 . \tag{21}$$

On expanding this, Equation (20) is obtained, with the added factor $\left(X_1-\frac{k}{X_2}\right)$. It is evident that in this form the alignment chart will have both the X_1 and the $\frac{k}{X_2}$ scales on the parabola $y=x^2$, and the $[f_3\phi_3]$ scale will lie on a curve defined by $x=-\frac{k}{F_3}$; $y=\frac{kG_3}{F_3}$. Since, however, the parabola is not suitable for practical use, two modifications of this determinant are used, one having a circle, and the other a hyperbola, take the place of the parabola.

To give the circular form we write $X_1=F_1$; $\frac{k}{X_2}=F_2$ for convenience and transform as follows:—

Equation (21) $\rightarrow$

$$\begin{vmatrix} \frac{F_3}{k}+G_3 & -2 & \frac{F_3}{k}-G_3 \\ 1+F_1^2 & 2F_1 & 1-F_1^2 \\ 1+F_2^2 & 2F_2 & 1-F_2^2 \end{vmatrix} \rightarrow \begin{vmatrix} \frac{F_3-kG_3}{F_3+kG_3} & \frac{-2k}{F_3+kG_3} & 1 \\ \frac{1-F_1^2}{1+F_1^2} & \frac{2F_1}{1+F_1^2} & 1 \\ \frac{1-F_2^2}{1+F_2^2} & \frac{2F_2}{1+F_2^2} & 1 \end{vmatrix} = 0 .$$

The conic is here defined by $x=\dfrac{1-F^2}{1+F^2}$; $y=\dfrac{2F}{1+F^2}$; whence $x^2+y^2=1$, the unit circle. The hyperbolic form may be obtained in a similar manner

$$\begin{vmatrix} \dfrac{F_3+kG_3}{F_3-kG_3} & \dfrac{-2k}{F_3-kG_3} & 1 \\ \dfrac{1+F_1^2}{1-F_1^2} & \dfrac{2F_1}{1-F_1^2} & 1 \\ \dfrac{1+F_2^2}{1-F_2^2} & \dfrac{2F_2}{1-F_2^2} & 1 \end{vmatrix} = 0 .$$

Here the conic is defined by $x=\dfrac{1+F^2}{1-F^2}$; $y=\dfrac{2F}{1-F^2}$, whence $x^2-y^2=1$, the rectangular hyperbola.

Using the circular form, the substitution $F_1=\tan\dfrac{\theta_1}{2}$ leads to the simple conditions $x=\cos\theta_1$; $y=\sin\theta_1$, which furnish an easy method of plotting values of the functions F_1 and F_2 on the circle. This may be done by drawing radii at angles θ_1 with the x axis, the points of intersection with the circle having the functional values $F_1=\tan\dfrac{\theta_1}{2}$. This has been done in Fig. 1, and values of F_1 (ϕ in the figure) at $\theta=0°$, 90°, etc., are readily verified.

For the hyperbolic form the substitution $F_1=\tanh\dfrac{\theta_1}{2}$ is used, whence $x=\cosh\theta_1$; $y=\sinh\theta_1$. This form is also shown in Fig. 1.

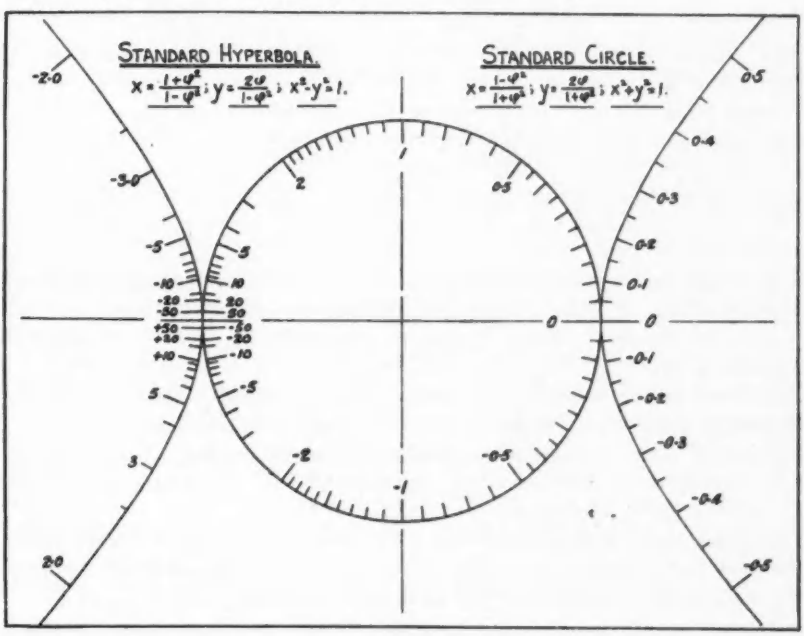

FIG. 1.

Of the two forms the circular is usually preferable because of its simple construction. However, in certain cases the third scale $[f_3\phi_3]$ may be found to lie at an inconvenient distance outside the circle. Whenever this is so, it will be found (no proof given here) that in the hyperbolic form the third scale will be between the two branches of the hyperbola.

The simplest method of studying the adjustment of scales is by using the parabolic form, and after the parabolic determinant has been transformed it may be put in either the circular or hyperbolic form.

We may write Equation (21) in the form

$$\begin{vmatrix} P_3 & Q_3 & R_3 \\ F_1 & F_1^2 & 1 \\ F_2 & F_2^2 & 1 \end{vmatrix} = 0 \qquad (22)$$

where the subscripts designate the three functions. Equation (21) was obtained by assuming $d \neq 0$. There are three similar forms for each of a, b, c, not zero. In each of the four cases we may obtain, by substituting back, the equivalents of P_3, Q_3, R_3, F_1, and F_2 in terms of the original constants and functions of the general equation. These equivalents are as shown in Table I.

TABLE I

ELEMENTS OF PARABOLIC FORM (22)

$a \neq 0$	$b \neq 0$	$c \neq 0$	$d \neq 0$
$P_3 = -(ad-bc)D_3$	$(ad-bc)C_3$	$(ad-bc)B_3$	$(ad-bc)A_3$
$Q_3 = (ad-bc)(aC_3-bD_3)$	$(ad-bc)(aC_3-bD_3)$	$-(ad-bc)(cA_3-dB_3)$	$-(ad-bc)(bA_3+dC_3)$
$R_3 = aB_3+cD_3$	bA_3+dC_3	aB_3+cD_3	cA_3-dB_3
$F_1 = \dfrac{(ad-bc)f_1}{a-cf_1}$	$\dfrac{(ad-bc)f_1}{df_1-b}$	$\dfrac{ad-bc}{a-cf_1}$	df_1-b
$F_2 = \dfrac{a+bf_2}{f_2}$	$a+bf_2$	$\dfrac{c+df_2}{f_2}$	$\dfrac{ad-bc}{c+df_2}$

It is found to be impossible to shift the scales along the conic independently of each other; in other words, overlapping portions of the two scales will remain overlapping. Equal values of the functions F_1 and F_2 appear as coincident points on the conic, and remain so. It is possible, however, to shift these points around the conic and in this way translate and magnify the scales together. The transformation in parabolic form is

$$\begin{vmatrix} mP_3+nR_3 & m^2Q_3+2mnP_3+n^2R_3 & R_3 \\ mF_1+n & (mF_1+n)^2 & 1 \\ mF_2+n & (mF_2+n)^2 & 1 \end{vmatrix} = 0. \qquad (23)$$

In the case of overlapping scales, very little may be done in a general way, the best plan being to try a few values of m and n and ascertain where the three scales lie. Each chart will be a study in itself.

In the case of non-overlapping scales, it may be found most suitable to arrange the conic scales symmetrically opposite each other. Suppose, for

instance, that we plot the extreme values of F_1 and of F_2 on the standard circle, denoting these end points of the scales by A, B, for F_1 and C, D, for F_2. Let us mark these so that in order around the circle they read $ABCD$. Then we must try to place A opposite C, and B opposite D.

To do this in our transformation, we have the conditions

$$(mA+n)(mC+n)=-1\ ;\ \ (mB+n)(mD+n)=-1.$$

Solving for m and n, this gives

$$m=\frac{A-B+C-D}{\sqrt{-(A-B)(B-C)(C-D)(D-A)}}$$

$$n=\frac{BD-AC}{\sqrt{-(A-B)(B-C)(C-D)(D-A)}}\ .$$

If it is found that this transformation places the third scale $[f_3\phi_3]$ at an inconvenient distance from the circle, the circular form of chart is still possible, as the $[f_3\phi_3]$ scale may be moved in closer to the circle. This is done by using a larger range of values for the variable which lies on the circle nearest the $[f_3\phi_3]$ scale. Thus we virtually reduce the working length of this circular scale, and the two conic scales will lie opposite each other, but one will be shorter than the other.

It is evident that in the determinant form the x column and the y column may be multiplied by any desired multiple, independently. This means that the abscissas and the ordinates in the chart may be plotted to any desired scale, not necessarily the same for both. In this way the conic scales may be plotted on an ellipse or on any desired hyperbola.

If such a procedure is intended in the case of the circular chart, and if it is found that the functional scales on the circle have their axis inclined at an angle α to the x axis, these scales should first be rotated until they are bisected by the x axis by using the substitutions $X=x\cos\alpha+y\sin\alpha$; $Y=y\cos\alpha-x\sin\alpha$, for all three scales in the circular determinant. Then all abscissas X and ordinates Y may be plotted according to independent scales, giving the elliptic form of chart. In the case of the hyperbolic chart this transformation will not be found necessary.

To summarize what has just been discussed:—

Type VII. Two scales on a circle and a curved scale.

The transformation of Equation (22) to Equation (23) with suitable values for m and n leads to the circular chart in which the functions f_1 and f_2 are represented by scales on the unit circle $\phi_1=mF_1+n$; $\phi_2=mF_2+n$, and the function $[f_3\phi_3]$ by the scale defined by $x=\dfrac{R_3(1-n^2)-2mnP_3-m^2Q_3}{R_3(1+n^2)+2mnP_3+m^2Q_3}$ and $y=\dfrac{2(mP_3+nR_3)}{R_3(1+n^2)+2mnP_3+m^2Q_3}$; the co-ordinates of the f_1 and f_2 scales are $x=\dfrac{1-\phi_1^2}{1+\phi_1^2}$; $y=\dfrac{2\phi_1}{1+\phi_1^2}$; and $x=\dfrac{1-\phi_2^2}{1+\phi_2^2}$; $y=\dfrac{2\phi_2}{1+\phi_2^2}$.

Rotation by an angle α about the origin will be obtained by finding new co-ordinates in each of the three functions using the substitutions

$$X = x\cos\alpha + y\sin\alpha;\quad Y = y\cos\alpha - x\sin\alpha.$$

Type VIII. Two scales on a hyperbola and a curved scale.

In the case of the hyperbolic chart the three scales are finally given by

$$x = \frac{1+\phi_1^2}{1-\phi_1^2};\quad y = \frac{2\phi_1}{1-\phi_1^2} \text{ for } f_1;\quad x = \frac{1+\phi_2^2}{1-\phi_2^2};\quad y = \frac{2\phi_2}{1-\phi_2^2} \text{ for } f_2;$$

and

$$x = \frac{R_3(1+n^2)+2mnP_3+m^2Q_3}{R_3(1-n^2)-2mnP_3-m^2Q_3};\quad y = \frac{2(mP_3+nR_3)}{R_3(1-n^2)-2mnP_3-m^2Q_3} \text{ for } [f_3\phi_3].$$

There follow some examples illustrating the preceding text.

Example 1. For a simply supported beam, as shown in Fig. 2, the shear at any section A is given by the formula

$$V = wl_1\left[\tfrac{1}{2}\left(\frac{x}{l_1}\right)^2 - \tfrac{1}{6}\frac{l_1}{l}\right] = Swl_1 .$$

The chart at the left side has been drawn to solve for S. If $\frac{l_1}{l}$ is denoted by a, and $\frac{x}{l_1}$ by b, then $S=\frac{1}{2}b^2-\frac{1}{6}a$. This is an equation of the third order already in the standard form $\alpha+\beta+\gamma=0$. Placing the a scale at the left

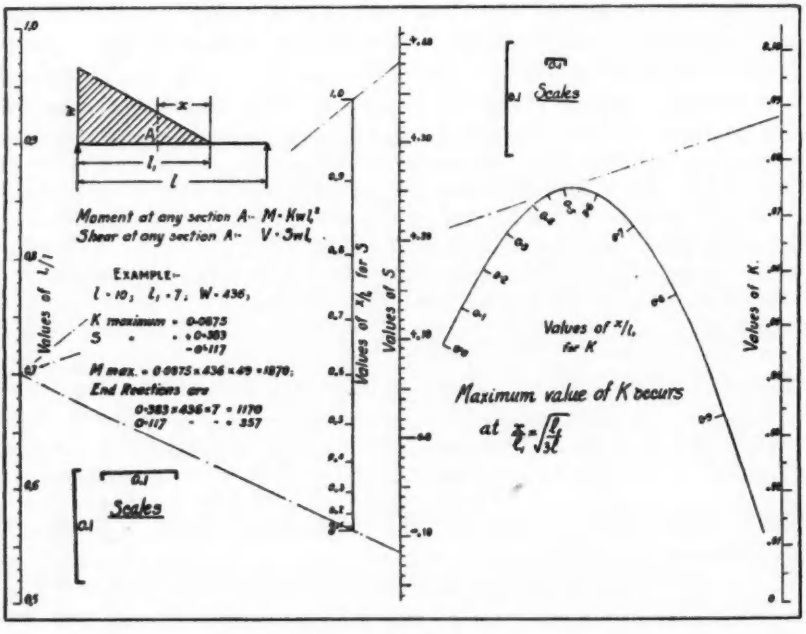

FIG. 2.

and the S scale at the right in a chart of Type I will require that $\alpha=\frac{1}{6}a$; $\gamma=S$; $\beta=-\frac{1}{2}b^2$.

Using a range for a from 0.5 to 1.0, it is evident from the equation that the extreme values for S are $+\frac{5}{12}$ at the left support when $\frac{l_1}{l}=0.5$, and $-\frac{1}{6}$ at the right support when $\frac{l_1}{l}=1.0$. For the α and γ scales to lie opposite each other we must have

$$\alpha_1 = m\gamma_1 + n \ , \quad i.e., \quad \frac{0.5}{6} = m(-\tfrac{1}{6}) + n$$

$$\alpha_2 = m\gamma_2 + n \ , \quad i.e., \quad \frac{1.0}{6} = m(\tfrac{5}{12}) + n \ .$$

Solving for m and n, $m=\frac{1}{7}$; $n=\frac{3}{28}$, and the final determinant, after multiplying all elements in the centre column by 6, becomes

$$\begin{vmatrix} 0 & a & 1 \\ 1.75 & 0.375\,b^2+0.5625 & 1 \\ 2 & \frac{8}{7}S+\frac{9}{14} & 1 \end{vmatrix} = 0 \ .$$

The resulting chart thus has the a or $\frac{l_1}{l}$ scale defined by $x=0$; $y=\frac{l_1}{l}$; the b or $\frac{x}{l_1}$ scale defined by $x=1.75$; $y=\frac{3}{8}\left(\frac{x}{l_1}\right)^2+\frac{9}{16}$; and the S scale defined by $x=2$; $y=\frac{8}{7}S+\frac{9}{14}$.

If it were desired to construct the chart with the S scale in the centre, then we must put $\alpha=\frac{1}{6}a$; $\gamma=-\frac{1}{2}b^2$; $\beta=S$. In this case we must equate extreme values of α and γ in the transformation, so that $\frac{1}{6}(0.5)=m(-\frac{1}{2}\times 1^2)+n$; $\frac{1}{6}(1.0)=m(0)+n$, whence $m=\frac{1}{6}$; $n=1$. In this chart $a=0.5$ lies opposite $b=1$, and $a=1.0$ lies opposite $b=0$. Note that the b scale is reversed when the S scale is placed in the centre. The determinant form here is

$$\begin{vmatrix} 0 & a & 1 \\ \frac{1}{7} & \frac{8}{7}(1-S) & 1 \\ 2 & 1-\frac{1}{2}b^2 & 1 \end{vmatrix} = 0 \ .$$

Example 2. For the same beam of Fig. 2 the bending moment at any section A is given by $M = wl_1^2\left[\frac{x+l-l_1}{6l}-\frac{x^3}{6l_1^3}\right]=Kwl_1^2$ and the chart at the right side solves for K.

Denoting as before $\frac{l_1}{l}$ by a and $\frac{x}{l_1}$ by b the formula becomes $K=\frac{1}{6}(ab+1-a-b^3)$. This is seen to be an equation of the fourth order and without taking the trouble to go through all the steps given in the text, we can by trial put it in the form $\alpha_1+\beta+\alpha_2\gamma=0$ as follows:—

$6K-1=ab-a-b^3=a(b-1)-b^3$, or $b^3+(6K-1)+(1-b)a=0$, and we may substitute $\alpha_1=b^3$; $\alpha_2=(1-b)$; $\beta=6K-1$; $\gamma=a$.

We have chosen in the chart to place the γ and β scales on the outside and the curved $[\alpha_1\alpha_2]$ scale in between. We must therefore choose the first

of the two transformations of Type V, since the range for $\alpha_2=(1-b)$ is 0 to 1 and hence $\frac{1}{1+\alpha_2}$ ranges between 1 and $\frac{1}{2}$.

The extreme limits of a are, as before, 0.5 and 1.0. The maximum value of K is found to occur at $a=0.5$ and $b=\sqrt{\frac{1}{6}}$, giving K maximum $=0.106$. (The proof of this involves an interesting study of the expression $ab+1-a-b^3$ which will not be given in detail here.) For γ and β scales opposite each other, extreme values are equated, giving

$$\gamma_1 = m\beta_1 + n \quad \text{or} \quad 0.5 = m(-1) + n$$
$$\gamma_2 = m\beta_2 + n \quad \text{or} \quad 1.0 = m(6+0.106-1) + n \ .$$

Hence $m=0.786$ and $n=1.286$, and the final determinant form is

$$\begin{vmatrix} 1 & \frac{1.286-0.786b^3}{1.786-0.786b} & 1 \\ 0 & a & 1 \\ 1 & 0.5+4.716K & 1 \end{vmatrix} = 0 \ .$$

If it were desired to place the K scale in the centre, the second of the two transformations of Type V should be used. Here it is necessary to arrange the straight a scale opposite the curved b scale, which is rather difficult. However, we may first place $a=1.0$ opposite $b=1.0$ and then arrange the constants m and n so that the lowest point of the curved b scale will be opposite $a=0.5$. The first condition, using $\gamma = \frac{n+m\alpha_1}{1-m\alpha_2}$, gives $1.0 = \frac{n+m}{1-0} = n+m$. The second condition is that the minimum value of $\frac{n+mb^3}{1-m(1-b)}$ shall be 0.5, for b lying between 0 and 1.

Substituting $n=1-m$ this gives us $\frac{1+m(b^3-1)}{1+m(b-1)} \geqq 0.5$, which may be written $\frac{1-m}{m} \geqq b(1-2b^2)$ or that $\frac{1-m}{m}$ shall equal the maximum value of $b(1-2b^2)$.

By differentiation the maximum of this expression occurs at $b=\sqrt{\frac{1}{6}}$, whence $\frac{1-m}{m}=\sqrt{\frac{1}{6}}(1-\frac{1}{3})$ and $m=0.786$. Hence $n=0.214$.

Another method of determining m and n would have been to place the greatest value of K (*i.e.*, 0.106) opposite the value of $a=0.5$. Thus

$$\gamma_1 = n - m\beta_1 \text{ or } 0.5 = n - m(0.636-1) \ ; \quad \gamma_2 = n - m\beta_2 \text{ or } 1 = n - m(-1);$$
$$\text{giving } m = 0.786 \ ; \quad n = 0.214 \text{ as before.}$$

The final determinant form would be

$$\begin{vmatrix} 1 & \frac{0.214+0.786b^3}{0.214+0.786b} & 1 \\ 0 & a & 1 \\ 1 & 1-4.716K & 1 \end{vmatrix} = 0 \ .$$

Example 3. For lack of a more common equation to illustrate Types VII and VIII, the historic formula of Massau's is chosen

$$(1+L)H^3 - LH(1+p) - \tfrac{1}{2}(1-L)(1+2p) = 0 \ .$$

Putting this in the general form (Equation 11), it becomes

$$3H^2(0+L+0+1) - 3H(Lp+L+0+0) + (2Lp+L-2p-1) = 0 \ .$$

On inspection none of the expressions A_3, B_3, C_3, D_3 are identically zero. We must therefore evaluate

$$a=\begin{vmatrix} 1 & 1 & 1 \\ 0 & 0 & -2 \\ 1 & 0 & -1 \end{vmatrix} = -2; \ b=\begin{vmatrix} 0 & 1 & 2 \\ 0 & 0 & -2 \\ 1 & 0 & -1 \end{vmatrix} = -2; \ c=\begin{vmatrix} 0 & 1 & 2 \\ 1 & 1 & 1 \\ 1 & 0 & -1 \end{vmatrix} = 0; \ d=\begin{vmatrix} 0 & 1 & 2 \\ 1 & 1 & 1 \\ 0 & 0 & -2 \end{vmatrix} = 2 .$$

Hence $ad-bc=-4$, and a conic chart is necessary.

Looking over the forms of Equation (22) in Table I we choose the one for $a \neq 0$, giving

$$P_3 = 4D_3 = 4(3H^2-1)$$
$$Q_3 = -4(-2C_3+2D_3) = -8(3H^2+1)$$
$$R_3 = -2B_3 = -2(3H^2-3H+1)$$
$$F_1 = \frac{-4f_1}{-2} = 2L$$
$$F_2 = \frac{-2-2f_2}{f_2} = -2\left(\frac{1+p}{p}\right) \ .$$

Using ranges of values of L from 0.5 to 1.0 and of p from 0.5 to 1.0, the corresponding limits of F_1 are 1 and 2, and F_2 ranges from -6 to -4. The four limiting points on the circle which are to be arranged symmetrically are $A=1$; $B=2$; $C=-6$; $D=-4$.

Before proceeding it may be as well to check up on the position of the H scale. It is found to have a range of values from $H=0.75$ to 1.0 and in the circular chart (before transformation) $x=\dfrac{R_3-Q_3}{R_3+Q_3}$; $y=\dfrac{2P_3}{R_3+Q_3}$ tells us that x varies from $-\frac{48}{176}$ to $-\frac{16}{17}$ and y from $-\frac{44}{176}$ to $-\frac{8}{17}$. The H scale therefore lies within the circle.

Proceeding with the transformation for symmetry of the conic scales,

$$m = \frac{A-B+C-D}{\sqrt{\text{etc.}}} = -\frac{3}{\sqrt{80}}; \quad n = \frac{BD-AC}{\sqrt{\text{etc.}}} = -\frac{2}{\sqrt{80}} \ .$$

Substituting these values in our summary for Type VII we find the ϕ_1 or L scale located by

$$x = \frac{1-(mF_1+n)^2}{1+(mF_1+n)^2} = \frac{19-6L-9L^2}{21+6L+9L^2}$$
$$y = \frac{2(mF_1+n)}{1+(mF_1+n)^2} = \frac{-20(3L+1)}{\sqrt{5}(21+6L+9L^2)} \ .$$

The ϕ_2 or p scale is located by

$$x = \frac{16p^2-12p-9}{24p^2+12p+9}; \quad y = \frac{20p(2p+3)}{\sqrt{5}\,(24p^2+12p+9)};$$

and the $[f_3\phi_3]$ scale located by

$$x = \frac{4.8H^2-5.7H+0.4}{7.2H^2-6.3H+3.6}; \quad y = \frac{2(6H^2+3H-4)}{0.9\sqrt{5}\,(8H^2-7H+4)} \ .$$

To determine the rotation of the scales, the range of values of $\phi_1 = mF_1 + n$ is found to be $-\frac{2.5}{\sqrt{20}}$ to $-\frac{4}{\sqrt{20}}$, and of $\phi_2 = mF_2 + n$ from $\frac{8}{\sqrt{20}}$ to $\frac{5}{\sqrt{20}}$. Using $\tan\frac{\theta}{2} = \phi$, this gives a range for θ_2 from 121° 35′ to 96° 23′, so that the angle of rotation $\alpha = 108°\ 59'$, and the required substitutions are

$$X = x\cos\alpha + y\sin\alpha = -.3253x + .9456y$$
$$Y = y\cos\alpha - x\sin\alpha = -.9456x - .3253y$$

The result is the chart shown in Fig. 3.

For the hyperbolic chart this last rotation is not necessary and the three scales are given by

$$x = \frac{21 + 6L + 9L^2}{19 - 6L - 9L^2}; \qquad y = \frac{-20(3L+1)}{\sqrt{5}(19 - 6L - 9L^2)} \text{ for } \phi_1 \text{ or } L$$

$$x = \frac{24p^2 + 12p + 9}{16p^2 - 12p - 9}; \qquad y = \frac{20p(2p+3)}{\sqrt{5}(16p^2 - 12p - 9)} \text{ for } \phi_2 \text{ or } p$$

$$x = \frac{7.2H^2 - 6.3H + 3.6}{4.8H^2 - 5.7H + 0.4}; \qquad y = \frac{2(6H^2 + 3H - 4)}{\sqrt{5}(4.8H^2 - 5.7H + 0.4)} \text{ for } [f_3\phi_3] \text{ or } H.$$

This chart is shown in Fig. 4.

Conic Charts for Third Order Equations

As mentioned before, all third order equations may be represented by nomograms in which two of the scales lie on a single conic and the third scale on a straight line. We have already shown that all such equations may be expressed in one or other of the canonical forms (*A*) $\alpha+\beta+\gamma=0$, (*B*) $\alpha+\beta\gamma=0$. However, in Class (*b*) when $P^2+B_1B_2B_3<0$, this reduction was possible only by the use of inverse tangents, and in this study of conic charts it is desirable to treat this Class (*b*) separately from the other two, and it will be called the transcendental type.

From Equation (5), which may be written $\tan^{-1}\alpha+\tan^{-1}\beta+\tan^{-1}\gamma=0$, we have directly that $\alpha\beta\gamma-(\alpha+\beta+\gamma)=0$ — (*D*) our third canonical form for third order equations. Here $\alpha=\frac{B_1X_1+P}{R_1}$; $\beta=\frac{B_2X_2+P}{R_1}$; $\gamma=\frac{R_1X_3}{PX_3-B_1B_2}$; but of course owing to symmetry the functions may be interchanged.

A study of Equations (20) and (21) shows that the symmetric equation

$$f_1f_2A_3+(f_1+f_2)B_3+C_3=0 \tag{24}$$

leads to the parabolic determinant

$$\begin{vmatrix} -B_3 & C_3 & A_3 \\ f_1 & f_1' & 1 \\ f_2 & f_2' & 1 \end{vmatrix} = 0\ . \tag{25}$$

For fourth order equations, the scale for $[f_3\phi_3]$ was curved because the functions in the top row of the determinant were not linearly connected. Here in the third order equations, owing to the linear relation between A_3, B_3 and C_3, this scale will be found to lie on a straight line.

(*1*). $\alpha+\beta+\gamma=0$ — (*C*). This may be put into the form of Equation (24)

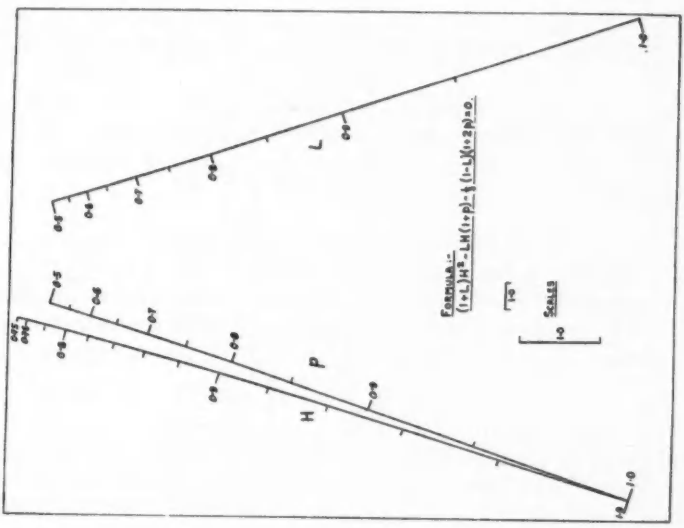

FIG. 4.

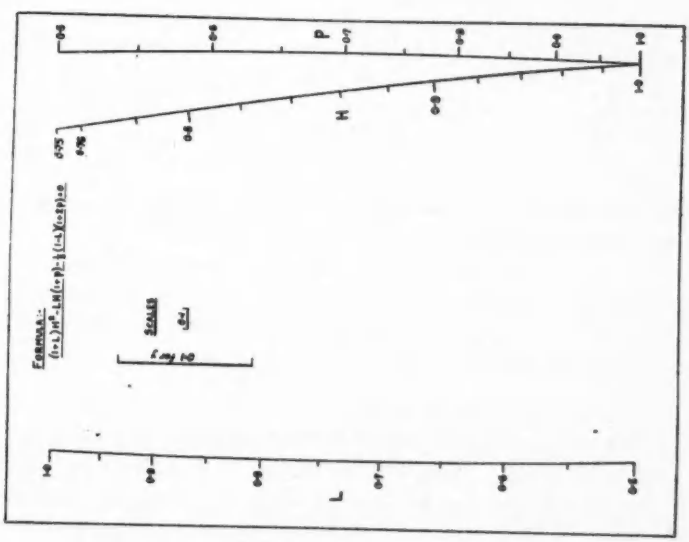

FIG. 3.

by making $A_3=0$, $B_3=1$, and $C_3=f_3$. In this case therefore Determinant (25) becomes

$$\begin{vmatrix} -1 & f_3 & 0 \\ f_1 & f_1^2 & 1 \\ f_2 & f_2^2 & 1 \end{vmatrix} = 0 .$$

representing $f_1+f_2+f_3=0$. Write $f_1=\beta$; $f_2=\gamma$; $f_3=\alpha$.

To shift the scales with respect to one another we may write the given canonical form $(m\beta+k)+(m\gamma+l)+(m\alpha-k-l)=0$ and the resulting chart will be indicated by

$$\begin{vmatrix} -1 & m\alpha-k-l & 0 \\ m\beta+k & (m\beta+k)^2 & 1 \\ m\gamma+l & (m\gamma+l)^2 & 1 \end{vmatrix} = 0 .$$

We have here chosen β and γ for the scales on the conic, and α on the straight line; m, k and l should be chosen so as to place the scales advantageously.

If the circular chart is chosen, the α scale is defined by $x=-1$; $y=\dfrac{-2}{m\alpha-k-l}$, which is tangent to the circle at $x=-1$.

It will be most convenient therefore to have the β and γ scales bisected by the x axis, *i.e.*, one passing through $\phi=0$ and the other through $\phi=\infty$.

In each particular example the ranges of the variables will determine the best adjustment, but the following pointers may not be amiss.

(*a*) If only one of the variables β,γ passes through the value ∞, the form given above is suitable as it stands.

(*b*) If neither β nor γ passes through the value ∞ the original equation should be written $(\alpha-p)+\beta+(\gamma+p)=0$, where p is so chosen as to make one of the conic scales pass through the value zero, and also ensure that the scales do not overlap.

The reciprocal form

$$\begin{vmatrix} -1 & 0 & \alpha-p \\ \dfrac{1}{\beta} & \left(\dfrac{1}{\beta}\right)^2 & 1 \\ \dfrac{1}{\gamma+p} & \left(\dfrac{1}{\gamma+p}\right)^2 & 1 \end{vmatrix} = 0$$

will then have the β and γ scales with one passing through $\phi=\infty$ and the other not. The final transformation for this is

$$\begin{vmatrix} k\alpha_1-m & k^2\alpha_1-2km & \alpha_1 \\ m\beta_1+k & (m\beta_1+k)^2 & 1 \\ m\gamma_1+k & (m\gamma_1+k)^2 & 1 \end{vmatrix} = 0$$

where we have written $\alpha_1=\alpha-p$; $\beta_1=\dfrac{1}{\beta}$; $\gamma_1=\dfrac{1}{\gamma+p}$.

Here m and k may be chosen to adjust the scales symmetrically.

(*c*) When both β and γ pass through the value infinity, the procedure of Case (*b*) may be followed. First, p is so chosen in $(\alpha-p)+\beta+(\gamma+p)=0$ that one of the conic scales does not pass through $\phi=0$ and the other does. Then in the reciprocal form both conic scales will pass through zero and only one through $\phi=\infty$. No values of m and k can prevent the scales from over-

lapping so that no suitable symmetric form is possible, even with the hyperbola. It is advisable in these rare cases either to construct two or more charts, each covering part of the range of values, or to replace one of the conic scales by α.

(2). $\alpha+\beta\gamma=0$ — (*B*). Substituting in Equation (24) $f_1=\beta$; $f_2=\gamma$; $A_3=1$; $B_3=0$, $C_3=\alpha$ gives the canonical form. Determinant (25) then becomes

$$\begin{vmatrix} 0 & \alpha & 1 \\ \beta & \beta^2 & 1 \\ \gamma & \gamma^2 & 1 \end{vmatrix} = 0 \ .$$

It is evident from the symmetry of the alternative form $\frac{1}{\alpha} \ . \ \beta \ . \ \gamma = -1$ that any two functions may be chosen for the conic scales. To effect the shifting of the scales with respect to one another the transformation

$$\begin{vmatrix} k & mn\alpha + k^2 & 1 \\ m\beta + k & (m\beta + k)^2 & 1 \\ n\gamma + k & (n\gamma + k)^2 & 1 \end{vmatrix} = 0$$

may be used.

In the circular chart the α scale is found to cut the circle. (In the fundamental form this α scale lies on the x axis.) Hence the circular form of chart will be most suitable, as in the hyperbolic form the α scale will lie to one side and not between the two branches. The most suitable method of determining m, n and k seems to be by trial. Note that a multiple m or n greater than unity shifts the scales towards the left of the circle, and a constant k positive moves the scales in a counterclockwise direction. As in Case (1, *c*) above, it is impossible to obtain suitable transformations when the β and γ scales cover very wide ranges of value (*e.g.*, through infinity). Such conditions seldom arise when accurate results are required, and charts covering such wide ranges are useful for general study and comparison only. Either the transformation given above, or its reciprocal,

$$\begin{vmatrix} k & 1 & mn\alpha + k^2 \\ \dfrac{1}{m\beta + k} & \left(\dfrac{1}{m\beta + k}\right)^2 & 1 \\ \dfrac{1}{n\gamma + k} & \left(\dfrac{1}{n\gamma + k}\right)^2 & 1 \end{vmatrix} = 0$$

should be sufficient to cover all practical cases. Otherwise, two or more charts should be used, or else one of the conic scales should be replaced by α.

(3). The transcendental type $\alpha\beta\gamma-(\alpha+\beta+\gamma)=0$ — (*D*).

For this case we substitute in Equation (24) $f_1=\beta$; $f_2=\gamma$; $A_3=\alpha$; $B_3=-1$; $C_3=-\alpha$. Hence Determinant (25) becomes

$$\begin{vmatrix} 1 & -\alpha & \alpha \\ \beta & \beta^2 & 1 \\ \gamma & \gamma^2 & 1 \end{vmatrix} = 0 \ .$$

To effect the transformation here both conic scales are first moved together, so that the β scale will be in its final position.

Thus

$$\begin{vmatrix} m + n\alpha & -m^2\alpha + 2mn + n^2\alpha & \alpha \\ m\beta + n & (m\beta + n)^2 & 1 \\ m\gamma + n & (m\gamma + n)^2 & 1 \end{vmatrix} = 0, \text{ which we write } \begin{vmatrix} -G_1 & H_1 & F_1 \\ \beta_1 & \beta_1^2 & 1 \\ \gamma_1 & \gamma_1^2 & 1 \end{vmatrix} = 0 .$$

Assume now that it is possible to use a transformation $\gamma_2=\frac{p+q\gamma_1}{r+s\gamma_1}$, where p, q, r, s are constants to be determined, so as to satisfy the condition of symmetry in β_1 and γ_2.

The assumption may be written $\gamma_1=-\frac{p-r\gamma_2}{q-s\gamma_2}$. On substituting this in the equation $\beta_1\gamma_1F_1+(\beta_1+\gamma_1)G_1+H_1=0$ we obtain

$$\beta_1\gamma_1(rF_1-sG_1)+\beta_1(qG_1-pF_1)+\gamma_2(rG_1-sH_1)+(qH_1-pG_1)=0 \tag{26}$$

For symmetry in β_1 and γ_2 the condition is $qG_1-pF_1=rG_1-sH_1$ which reduces to $(q-r)(m+n\alpha)+p\alpha-s(-m^2\alpha+2mn+n^2\alpha)\equiv 0$.

Hence $(q-r)n+p+(m^2-n^2)s=0$; $q-r-2ns=0$.

These two conditions reduce to $p=-(m^2+n^2)s$ and $q=r+2ns$.

Substitute now for p and q, F_1, G_1, and H_1 in Equation (26), and put $R=r+ns$; $S=ms$ and the symmetric form $\beta_1\gamma_2[R\alpha+S]+(\beta_1+\gamma_2)[(mS-nR)\alpha-(mR+nS)]+[(n^2R-m^2R-2mnS)\alpha+(n^2S-m^2S+2mnR)]=0$ results.

Making $S=$ unity does not interfere with the generality, and we have the final parabolic form

$$\begin{vmatrix} (nR-m)\alpha+(mR+n) & [(n^2-m^2)R-2mn]\alpha+2mnR+n^2-m^2 & R\alpha+1 \\ m\beta+n & (m\beta+n)^2 & 1 \\ \dfrac{(mR+n)\gamma+(nR-m)}{R+\gamma} & (\gamma \text{ function})^2 & 1 \end{vmatrix}=0. \tag{27}$$

Here m and n may be chosen first, then R, to give suitable positions. Note that in this case the α scale lies outside the circle and between the branches of the hyperbola.

The transformations given in Cases (1) and (2) are particular cases given by the method which has just been used in Case (3), but owing to the simple nature of the canonical forms, it was possible to find them by much simpler routes. The various cases of the general third order equation (seven of them) may also be studied by this method, but such a procedure proved tedious and complicated. The study given above, of the three canonical forms, to which they can all be reduced, has been found preferable.

Example 4. $\text{Tan}\,(P+Q)=\dfrac{\tan P+\tan Q}{1-\tan P\tan Q}$ illustrates Case (3) above.

This may be written $-\tan(P+Q)\tan P.\tan Q-(\tan P+\tan Q-\tan\overline{P+Q})=0$, evidently of canonical type (D).

If we use the circular chart (Fig. 5) and assume a range of values for both $\tan P$ and $\tan Q$ from 0 to 1, and place the $\tan P$ scale on the right, extending from $\phi=-\frac{1}{4}$ to $\phi=+\frac{1}{4}$, we have the conditions (see Equation (27)) $m.0+n=-\frac{1}{4}$; $m.1+n=+\frac{1}{4}$, whence $m=\frac{1}{2}$; $n=-\frac{1}{4}$.

Choosing now a symmetrical position for the Q scale at the left we must use the condition

$$\frac{(mR+n)0+(nR-m)}{R+0}=-\frac{(mR+n)1+(nR-m)}{R+1}.$$

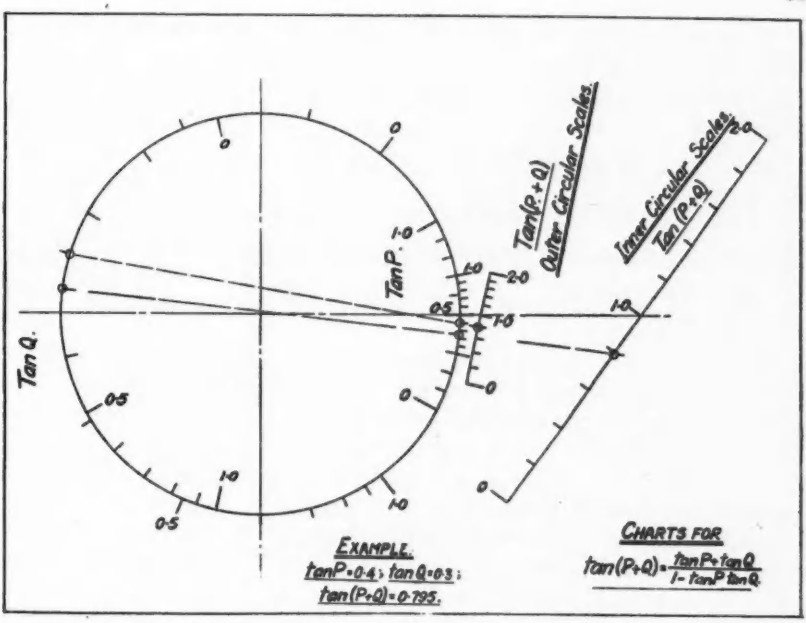
Tan Q.
Tan P.
Tan(P+Q)
Outer Circular Scales.
Inner Circular Scales.
Tan(P+Q)
EXAMPLE.
tanP=0·4; tanQ=0·3;
tan(P+Q)=0·795.
CHARTS FOR
tan(P+Q)=(tanP+tanQ)/(1-tanP tanQ)

FIG. 5.

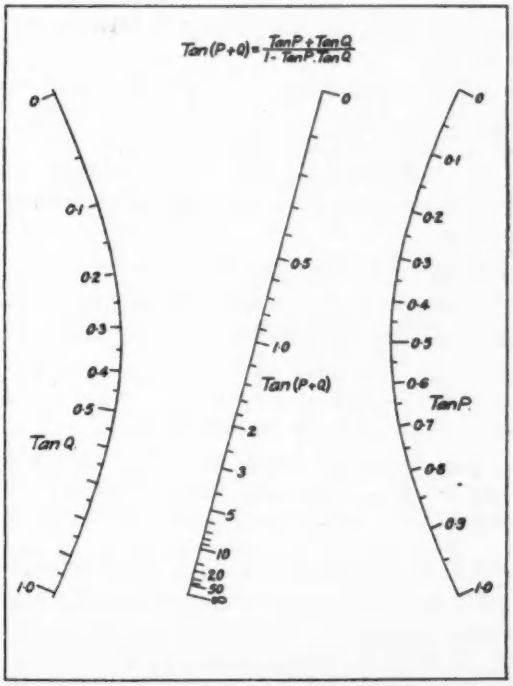
Tan(P+Q)=(TanP+TanQ)/(1-TanP.TanQ)
Tan Q.
Tan(P+Q)
Tan P.

FIG. 6.

Clearing and giving m and n their values, this makes $R=-\frac{1}{3}$. Equation (27) then reduces to

$$\begin{vmatrix} \frac{1}{4}(1-\tan\overline{P+Q}) & \frac{1}{12}(3\tan\overline{P+Q}+1) & -\tan(P+Q)-3 \\ \frac{1}{3}\tan P-\frac{1}{4} & \text{Square} & 1 \\ \frac{1}{4}\dfrac{\tan Q+1}{1-3\tan Q} & \text{Square} & 1 \end{vmatrix} = 0$$

from which we obtain the circular chart with the two scales printed on the inside of the perimeter. The $\tan(P+Q)$ scale is plotted as $x=\dfrac{31\tan(P+Q)+53}{\tan(P+Q)+43}$; $y=\dfrac{40\tan(P+Q)-40}{\tan(P+Q)+43}$; and is the outer of the two straight scales.

The use of a standard circular chart drawn the same size as the final chart and on which is marked accurately a complete ϕ scale, will be found to aid the plotting. This standard chart may be placed under a tracing cloth and the final chart plotted on the tracing.

The second set of scales shown in the figure may be obtained in a similar manner, but a much simpler way is to use the parabolic form of the first chart, multiplying the ϕ or x column by $\frac{2}{3}$ and the ϕ^2 or y column by $\frac{4}{25}$. In this way the tan P scale is reduced and the tan Q scale enlarged, the straight scale being brought in closer.

Fig. 6 illustrates the hyperbolic form for this equation, the straight scale lying between the branches of the conic. The inclination of this scale from the vertical is due to a slight lack of symmetry between the two conic scales.

Cubic Charts for Third Order Equations

Although this type of chart is not suitable for practical purposes, it is included here for the sake of completeness. Just as the conic charts are based on the idea of symmetry of two of the variables, so the cubic charts are based on symmetry of all three variables, the three scales appearing on a single (cubic) curve.

All third order equations may be put in the form

$$\phi_1\phi_2\phi_3 + A(\phi_1\phi_2+\phi_2\phi_3+\phi_3\phi_1) + B(\phi_1+\phi_2+\phi_3) + C = 0 \qquad (28)$$

and this leads to the determinant form

$$\begin{vmatrix} \phi_1+A & \phi_1^2-B & \phi_1^3+C \\ \phi_2+A & \phi_2^2-B & \phi_2^3+C \\ \phi_3+A & \phi_3^2-B & \phi_3^3+C \end{vmatrix} = 0 \ . \qquad (29)$$

Expanding Equation (29) gives Equation (28) with three factors $(\phi_1-\phi_3)(\phi_2-\phi_3)(\phi_3-\phi_1)$. This determinant evidently gives a chart with all three variables on the same curve. If the first column is made unity, the curve is defined by $x=\dfrac{\phi^3-B}{\phi+A}$; $y=\dfrac{\phi^3+C}{\phi+A}$. On eliminating ϕ the equation $(C-A^3)x^3+(B-A^2)(x^2-y)y+(C-AB)(3Ax-y+3B)x-2(AC-B^2)y+(C^2-B^3)=0$ is obtained.

Similar equations result from making the other columns unity.

Let us now study each of the three canonical forms.

(*i*). $\phi_1+\phi_2+\phi_3=0$. For this form we substitute in Equation (28) $A=C=0$; $B=\infty$, giving the determinant

$$\begin{vmatrix} \phi_1 & 1 & \phi_1^2 \\ \phi_2 & 1 & \phi_2^2 \\ \phi_3 & 1 & \phi_3^2 \end{vmatrix} = 0 \ .$$

Making in turn each of the three columns unity here we obtain the three cubic charts

$$(a) \quad x=\frac{1}{\phi}\ ; \quad y=\phi^2\ ; \quad x^2y=1\ ;$$

$$(b) \quad x=\phi\ ; \quad y=\phi^3\ ; \quad x^3=y\ ;$$

$$(c) \quad x=\frac{1}{\phi^2}\ ; \quad y=\frac{1}{\phi^3}\ ; \quad x^3=y^2\ .$$

For these fundamental forms see Fig. 7 (i).

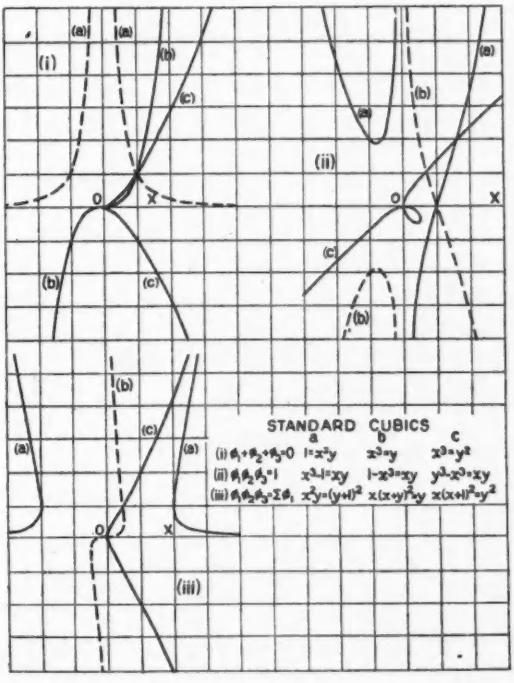

FIG. 7.

It is evident that the scales may be shifted with respect to one another by re-writing the canonical form $(m\phi_1+k)+(m\phi_2+l)+(m\phi_3-k-l)=0$.

(*ii*). $\phi_1\phi_2\phi_3=1$, which corresponds to $\alpha+\beta\gamma=0$.

Here substitute in Equation (28) $A=B=0$; $C=-1$ and the determinant becomes $\begin{vmatrix} \phi_1 & \phi_1^2 & \phi_1^3-1 \\ \phi_2 & \phi_2^2 & \phi_2^3-1 \\ \phi_3 & \phi_3^2 & \phi_3^3-1 \end{vmatrix}=0$.

By making in turn each of the three columns unity the three cubics are found to be

$$(a)\quad x=\phi\,; \qquad y=\frac{\phi^3-1}{\phi}\,; \qquad x^3-1=xy$$

$$(b)\quad x=\frac{1}{\phi}\,; \qquad y=\frac{\phi^3-1}{\phi^3}\,; \qquad 1-x^3=xy$$

$$(c)\quad x=\frac{\phi}{\phi^3-1}\,; \qquad y=\frac{\phi^2}{\phi^3-1}\,; \qquad y^3-x^3=xy\ .$$

See Fig. 7 (ii). In this case the scales may be shifted by re-writing the given form $(l\phi_1)\,.\,(m\phi_2)\left(\frac{\phi_3}{lm}\right)=1$. Other transformations are possible but it is not worth while to study them here.

(iii). $\phi_1\phi_2\phi_3-(\phi_1+\phi_2+\phi_3)=0$. In Equation (28) put $A=C=0$; $B=-1$, giving the determinant $\begin{vmatrix} \phi_1 & \phi_1^2+1 & \phi_1^3 \\ \phi_2 & \phi_2^2+1 & \phi_2^3 \\ \phi_3 & \phi_3^2+1 & \phi_3^3 \end{vmatrix}=0$.

Making in turn each of the three columns unity, we obtain

$$(a)\quad x=\frac{\phi^2+1}{\phi}\,; \qquad y=\phi^3\,; \qquad x^3y=(y+1)^2\,;$$

$$(b)\quad x=\frac{\phi}{\phi^2+1}\,; \qquad y=\frac{\phi^3}{\phi^2+1}\,; \qquad x(x+y)^2=y\,;$$

$$(c)\quad x=\frac{1}{\phi^2}\,; \qquad y=\frac{\phi^2+1}{\phi^3}\,; \qquad x(x+1)^2=y^2\ .$$

See Fig. 7 (iii). The transformation for shifting is more difficult here. One good method, which will be used, is to write the equation in the form $\tan^{-1}\phi_1+\tan^{-1}\phi_2+\tan^{-1}\phi_3=0$, whence $(\tan^{-1}\phi_1+\tan^{-1}l)+(\tan^{-1}\phi_2+\tan^{-1}m)+(\tan^{-1}\phi_3-\tan^{-1}l-\tan^{-1}m)=0$ is obtained directly.

This reduces to $\tan^{-1}\dfrac{\phi_1+l}{1-l\phi_1}+\tan^{-1}\dfrac{\phi_2+m}{1-m\phi_2}+\tan^{-1}\dfrac{(1-lm)\phi_3-(l+m)}{(1-lm)+(l+m)\phi_3}=0$,

which may be written $F_1F_2F_3=F_1+F_2+F_3$, where

$$F_1=\frac{\phi_1+l}{1-l\phi_1}\,; \qquad F_2=\frac{\phi_2+m}{1-m\phi_2}\,; \qquad F_3=\frac{(1-lm)\phi_3-(l+m)}{(1-lm)+(l+m)\phi_3}\ .$$

Example 5. We use once more the formula for $\tan(\alpha+\beta)$, choosing for its representation the cubic (iii *b*), $x(x+y)^2=y$. We put $\phi_1=\tan\alpha$, $\phi_2=\tan\beta$, and $\phi_3=-\tan(\alpha+\beta)$. In the final transformation, we have decided on $l=0.8$, $m=-9$. This gives

$$F_1=\frac{\tan\alpha+0.8}{1-0.8\tan\alpha}\,; \qquad F_2=\frac{\tan\beta-9}{1+9\tan\beta}\,; \qquad F_3=\text{etc.}=\frac{1-\tan(\alpha+\beta)}{1+\tan(\alpha+\beta)}\ .$$

The scales on the curve are marked off according to the co-ordinates $x=\dfrac{F}{F^3+1}$; $y=\dfrac{F^2}{F^3+1}$.

After making up a table of co-ordinates for the curve from $F=-9$ to $F=+9$, which are the extreme values, it appears advisable to use oblique axes. This may be done by keeping the same abscissas, but using new ordinates $Y=y-15x$, where (x,y) are the old co-ordinates. In this way better intersections are obtained for solutions. In the final form of the chart, the scale for abscissas has been magnified in order to increase the width of the chart (Fig. 8).

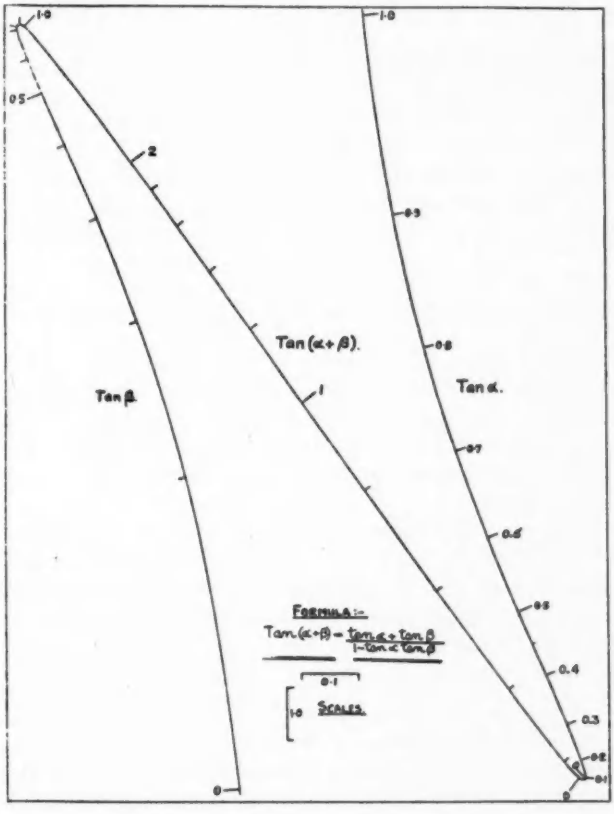

FIG. 8.

Inspection of this chart demonstrates that the cubic is not a practical form. It might be possible to adjust the values of l and m slightly and thus separate the α and $(\alpha+\beta)$ scales, and remove the ends of the three scales from the sharp bends of the curve, but this further adjustment would spread out the tan α scale near the value 1, and the tan β scale near the value 0, and correspondingly close up these scales at the other ends. Of course there are other transformations possible, but practical considerations do not seem to warrant much further study along these lines.

In conclusion, it is interesting to note that the three canonical forms which we have studied may be transformed into one another by the use of either logarithmic or transcendental transformations. Thus $\alpha\beta\gamma=1$ reduces to $\alpha+\beta+\gamma=0$ by taking logarithms, $\alpha\beta\gamma=\alpha+\beta+\gamma$ reduces to $\alpha+\beta+\gamma=0$ by using inverse tangents. The reverse processes are also possible.

A discussion of elementary determinant theory and its application to alignment charts will be found in a previous paper by the author (9, p. 374.)

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THE SORPTION OF VAPORS BY ACTIVE SILICA[1]

By L. M. Pidgeon[2]

Abstract

The sorptive properties of a new active silica have been examined. This sorbent is prepared by the action of acids on the mineral serpentine. The quartz spiral sorption balance has been employed to obtain isotherms for water, benzene and alcohol. Relative rates of sorption have been measured and compared with those for silica gel.

Efficiency measurements have also been made, using the dynamic method, with water vapor as the sorbate. Comparative measurements have been carried out on commercial silica gel, and data are cited from the literature for activated alumina. A comparison of the data indicates that active silica is inferior to silica gel but somewhat better than commercial alumina. Its simple method of production combined with reasonably good sorptive properties should result in a cheap and effective sorbent for technical purposes.

The type of isotherm exhibited by active silica is similar to that associated with the "chalky" gels described by Holmes. Certain theoretical aspects of sorption by "chalky" and "vitreous" gels are discussed.

Introduction

A large number of oxides may be obtained in a porous condition and in this state are known to be good sorbents of vapors and gases. These oxides obtain their porosity from the fact that they are produced by dehydration of the corresponding hydroxide which is generally a colloidal substance. Typical sorbents of this nature are alumina, titania, silica gel, etc. There is, however, another manner in which porosity may be produced but which is not directly connected with such colloidal structure. If the molecules of one component of a complex mixture, such as a naturally occurring silicate, are removed, and the remaining molecules still retain the same position relative to one another, a porous structure is formed which is not a dehydrated gel, yet which exhibits many of its characteristic properties. A well known example of this type of substance is presented in a dehydrated zeolyte such as chabasite, where the water of crystallization has been removed without altering the form of the compound. The resulting structure is enormously porous and capable of a very high degree of sorption.

D. Wolochow has developed in these laboratories a method which results in the production of a similar sorbent from the mineral serpentine. This mineral, which has the empirical formula $3\ MgO.2SiO_2.2H_2O$, when acted upon by dilute mineral acids loses the MgO and water of crystallization almost quantitatively, leaving only the silica and certain naturally occurring impurities. The essential point of the process is that, in the case of serpentine at least, the mineral does not lose its form during the treatment, so that it may be assumed that the silica molecules have the same spatial arrangement after treatment as they had before treatment. The removal of the other

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constituents of the mineral leaves innumerable submicroscopic spaces whose walls present a very large surface.

Preliminary experiments carried out with water at the saturation point indicated a very satisfactory degree of sorptive power, and a more extensive study seemed to be warranted. This paper deals with an examination of the sorptive properties of this substance to which the name "active silica" has been given.

Experimental

The sorption of water, benzene and alcohol vapors has been measured, using a modification of the sorption balance developed by McBain and Bakr (7). This method of measurement presents a number of advantages which need not be enumerated here.

Since the technical interest in this substance lies in its possible applications to commercial processes, a number of experiments have been carried out using the dynamic sorption method, as it is analogous to the conditions holding in the practical application of sorbents in the recovery of volatile solvents and in the drying of air.

I. Static Method

Apparatus. The apparatus is illustrated in Fig. 1, and is in general similar to that described previously (12). All stopcocks were replaced by mercury seals so that organic vapors could be examined. The spirals were mounted as shown in tubes *A*, *B* and *C* which were equipped with mercury-sealed ground glass connections; these were employed successfully in the dry condition. The tubes were immersed as shown in a thermostat, the temperature of which was maintained constant to within 0.02° C.

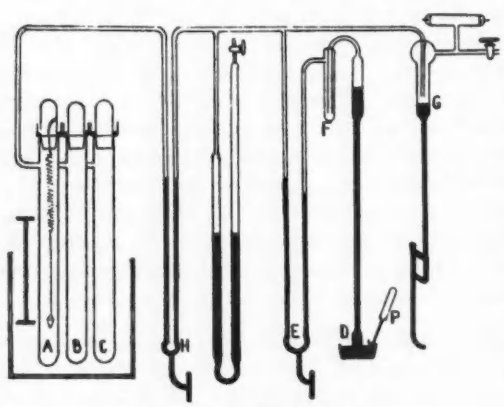

FIG. 1. *Sorption balance.*

The other parts of the apparatus are clearly shown in the diagram. The evacuating system consisted of the usual mercury diffusion pump backed by an oil pump. With this combination it was possible to reduce the pressure in the system to the limit of an ordinary McLeod gauge.

The vapor to be examined was added to the apparatus as a liquid at *D*. A special pipette, *P*, was employed in which the liquid was boiled to expel dissolved gases and then introduced under the open end of *D*; as the apparatus had been previously evacuated the liquid rose to the top of the mercury

column saturating the space *F* with vapor. It was then added to the sorption system as required by opening the cut-off *E*. The liquid could also be frozen and evacuated in the ordinary manner by immersing the tube *F* in a suitable freezing mixture. When a different sorbate was to be employed any liquid adhering to the walls of *D* was removed by gentle heating with a free flame during evacuation.

The quartz spirals were constructed by winding suitable fibres on a fluted carbon rod. A flame was used to soften the fibre at the appropriate point. The average spiral was 1.2 cm. in diameter and consisted of 50 turns. Weights up to 1.5 gm. could be supported, giving a total deflection of approximately 60 mm. Cathetometer readings were accurate to within 0.02 mm.

The samples under examination were placed in buckets constructed of aluminium gauze. The buckets were suspended from the spirals by means of a fine aluminium wire. The elongation caused by these additions was subtracted from the total to obtain the net elongation due to the sample.

Samples. All the samples of active silica which have been examined were prepared by D. Wolochow. They were obtained from serpentine (asbestos tailings) by treatment with hydrochloric or sulphuric acid. The screen sizes of the rock which were used varied from minus 8 to plus 16 mesh. The product is a chalky white substance containing occasional colored impurities. It is relatively soft and may be crushed by hand. The results of an analysis of a well prepared sample are shown in Table I.

TABLE I

RESULTS OF ANALYSIS OF A TYPICAL SAMPLE OF ACTIVE SILICA*

Treatment	Percentages, dry weight basis (ignition)				
	SiO_2	Al_2O_3	F_2O_3	CaO	MgO
H_2SO_4—1 : 3, 6 hr. boiling. Recovery, 44.5%	96.3	1.4	1.0	Trace	1.2

Procedure. The samples were placed in position as shown in the diagram and the whole apparatus evacuated until no further gas was given off by the samples. During this time they lost volatile sorbed gases and vapors and after about eight hours had reached a constant "dry" deflection which was ascertained by means of a cathetometer (both the upper and lower ends of the spiral were read on each occasion to obviate errors consequent on displacement of cathetometer or apparatus).

This "dry" weight is, of course, purely a relative one, but as it is readily reproducible it was chosen in the interests of convenience (see "Discussion of dry weight"). Cut-off *G* was then closed and *E* opened for a time, allowing

* *Analysis by C. W. Davis, Division of Chemistry, National Research Laboratories, Ottawa.*

vapor to enter the apparatus. *E* was closed, and after a time the pumping system was again operated and the samples were evacuated by opening *G*. This sequence of events was repeated at least three times and resulted in the "washing" out of inert sorbed gases such as air which might have interfered with the establishment of equilibrium. Vapor was then allowed to enter the apparatus until some suitable pressure was attained, after which the reaction system was closed and allowed to stand until the manometer indicated a constant pressure; readings were then taken with the cathetometer, as previously described.

In this form of the apparatus the pressure in the system falls during the establishment of equilibrium owing to the removal of vapor by the sorbent. These changes are, however, quite small, owing to the large volume of the apparatus compared with the size of samples, and as this fall in pressure is common to most standard sorption methods, the effect has been neglected. For the examination of hysteresis effects, however, it is desirable that the pressure should remain at a reasonably constant value during sorption or desorption at any point; hence a few experiments have been carried out in a previously described apparatus (12) in which these conditions are fulfilled.

Sorption of Water Vapor

1. *The sorption isotherm.* Typical values for the sorption of water vapor by active silica are shown in Table II. These results could be readily duplicated with any given sample although small variations existed among various samples of the same kind.

TABLE II

SORPTION OF WATER BY ACTIVE SILICA

Sample 81; temp., 23° C.		Sample 81; temp., 28° C.		Temp., 20° C. Sample 55		Sample 49
Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100	$\frac{p}{p_o}$. 100	x/m . 100	x/m . 100
Sorption		*Sorption*		*Sorption*		
8.6	3.0	5.9	2.8	5.3	2.4	2.4
19.7	5.4	16.2	4.9	21.1	4.6	5.4
27.6	7.1	29.3	7.1	41.3	7.3	7.8
41.0	9.3	37.1	8.6	50.0	8.5	9.1
52.8	11.8	47.3	10.5	67.8	11.5	11.7
76.2	17.1	61.1	13.5	82.2	15.3	17.3
95.2	22.8	67.1	15.5	100.0	17.0	19.9
Desorption		*Sorption*		*Desorption*		
100.0	23.8	4.0	2.6	85.1	15.7	18.3
93.4	22.3	23.7	6.2	76.0	14.2	16.1
79.5	18.4	33.1	7.4	61.4	11.5	12.6
61.3	14.0	44.5	10.3	52.8	10.3	10.5
49.0	10.9	55.8	12.3			
31.0	7.2	66.9	14.9			
24.6	5.6	84.0	20.2			
15.1	3.7	100.0	24.6			

One set of values is plotted in Fig. 2, where it produces the familiar sigmoid isotherm which characterizes the sorption of water vapor by a number of well known colloidal substances, such as cellulose, gelatin, etc.

Under the pressure conditions existing in these experiments it was possible to obtain reversible isotherms as seen in Fig. 2. In some experiments, however, there were indications of hysteresis, and, as will be seen later, when the pressure during sorption was not allowed to rise above the final equilibrium value at any point a hysteresis appeared.

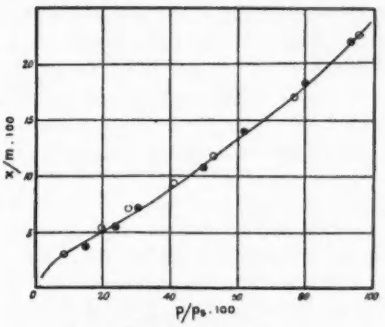

FIG. 2. *Sorption of water by active silica; isotherm at 23° C.*

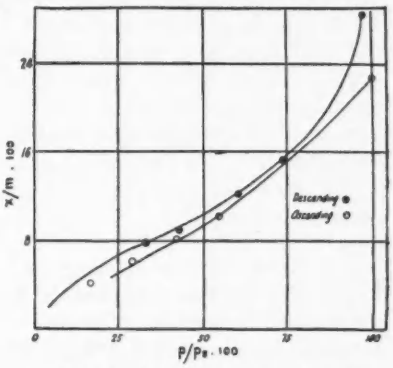

FIG. 3. *Sorption of water by active silica at 20° C.*

Between 20° and 28° C. no difference in sorption is apparent when the results are plotted against relative humidity.

With this type of isotherm, relatively high sorption values should be reached near the saturation pressure owing to the fact that in this region $d(x/m)/dp$ is increasing rapidly. There was evidence of incomplete attainment of equilibrium by sorption near the saturation pressure, as shown by the following experiment. The active silica was wetted with water and the desorption results obtained without preliminary drying. The results of this procedure are listed in Table III and plotted in Fig. 3. The high values which are shown near the saturation pressure are not reached on the subsequent sorption, so that the S-shaped curve is much more obvious when water is being lost than it is when water is being sorbed.

TABLE III

SORPTION OF WATER VAPOR BY ACTIVE SILICA (IN THE WET CONDITION INITIALLY) AT 20° C.

Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100
Sample containing free water		Sample dried	
Desorption		*Sorption*	
100.0	92.5	16.7	4.1
98.5	41.5	28.7	6.3
97.1	28.4	42.5	8.1
74.1	15.2	54.4	10.1
61.0	12.2	100.0	22.7
43.2	9.2		
33.0	7.8		

The shape of the isotherm is similar to that found in the case of alumina by other workers and similar to that found by Holmes and co-workers in the case of "chalky gels." It differs from the silica gel isotherm which approaches a definite saturation value for sorption at some relative humidity between 60 and 80%.

2. *Hysteresis.* It has been pointed out previously (12) that if hysteresis occurs, its true dimensions should be apparent only if the system as a whole approaches each sorption point strictly in the direction indicated. This result may be obtained only when the pressure in the system during sorption does not rise above that of the new equilibrium point. No simple methods are available to achieve this end, and as the principal aim of this work was to examine the magnitude of sorption by samples of active silica which had been produced by different treatments, the more direct methods of sorption measurement have been followed in most cases. One set of experiments in which the vapor pressure was kept at a constant value during sorption has been performed.

The same methods as those used previously in the case of silica gel were employed. In the first method the vapor pressure in the apparatus was maintained by placing a sulphuric acid solution of known strength in the system. In the second, a body of water in the sorption system was maintained at some constant temperature lower than that of the samples.

In order to obtain comparative results with the same sample under circumstances in which the pressure varied during sorption, a run was carried out in the ordinary manner. That is, the pressure in the system at the moment of addition of vapor was higher than the subsequent equilibrium point, hence sorption took place during a falling vapor pressure. This sequence of events is, of course, reversed during desorption. In order to make the pressure changes more acute a small amount of silica gel was placed in one tube, as the sorption balance, owing to the small size of the samples, shows a lower ratio of sorbent to the volume of system than is common in most other methods.

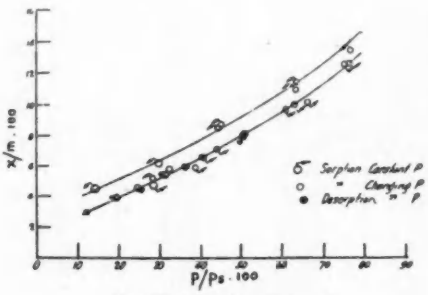

FIG. 4. *Sorption hysteresis.*

Results of this experiment appear in Table IV and Fig. 4, where it will be seen that a hysteresis appears when the vapor pressure is constant, while on the other hand, when fairly large variations appear, the isotherm is reversible. It was unfortunately impossible to continue the hysteresis experiments, hence the results are not sufficiently complete to discuss in detail.

TABLE IV

SORPTION WITH CONSTANT VAPOR PRESSURE

Sorption with constant vapor pressure; temp., 20.38° C.; Sample 32				Sorption with variable vapor pressure; temp., 20.44° C.; Sample 32					
Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100
Method 1		Method 2							
Sorption		*Sorption*		*Sorption*		*Desorption*		*Sorption*	
29.0	4.9	38.4	5.8	24.6	4.4	49.5	7.6	19.7	3.9
44.1	7.1	40.8	6.5	31.4	5.4	40.8	6.6	32.3	5.9
63.4	10.0	60.8	9.7	36.2	5.8	36.1	5.8	50.2	8.1
76.6	12.6	66.1	10.2	50.2	7.7	30.5	5.6	85.9	14.5
Desorption		75.1	12.5			25.2	4.5		
76.7	13.4	*Desorption*				18.2	3.7		
63.5	10.9	63.4	11.5			12.1	2.9		
44.4	8.5	45.4	8.7						
29.9	6.2	28.6	5.2						
14.2	4.4								

3. *Relative rate of sorption.* The relative rate of sorption in the static apparatus is measured by exposing the dried sorbent to the vapor under consideration at some constant pressure, and measuring the elongation of the spiral at various time intervals until no further change is noticed.

The true rate of sorption cannot be measured in this manner as the measured rate is partly dependent on the speed at which vapor may be evaporated from the liquid surface, and on the time taken for the vapor to penetrate into the lumps of sorbent. The measured rate is therefore a relative value and comparative standards are required to give meaning to the results. Commercial silica gel of about the same particle size has therefore been chosen and examined in the same manner as active silica.

The results of typical experiments of this nature carried out on these substances are shown in Table V.

TABLE V

RELATIVE RATE OF SORPTION

Active silica (No. 23)		Silica gel	
x/m . 100	Time, min.	x/m . 100	Time, min.
1.7	2.3	1.3	1.0
1.8	4.3	2.0	2.5
2.8	7.0	2.2	4.0
3.5	14.5	2.6	7.0
4.0	19.5	3.9	11.0
4.8	31.0	5.8	25.0
5.6	55.0	9.5	57.0
5.7	87.0	11.6	117.0
5.7	100.0	12.4	177.0
		13.9	328.0
		14.4	Final equilibrium

NOTE: *Temperature of thermostat, 20° C.; relative vapor pressure, 26.4%.*

Fig. 5 shows the rate of sorption as obtained in the static apparatus. It is to be observed that for approximately the first eight minutes the two sorbents take up water at almost the same rate. Active silica rapidly attains a saturation value after the lapse of an hour, while silica gel continues to sorb for

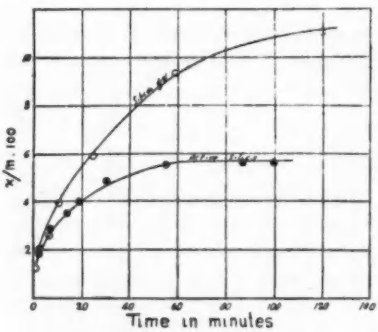

FIG. 5. *Relative rate of sorption at 20° C.; $p/p_s \cdot 100 = 26.4$.*

several hours, reaching a much higher final value. This difference in behavior suggests that active silica presents a much more open structure and one in which the innermost parts are readily accessible to the entering vapor. On the other hand, the surface must be greater in the case of silica gel, or at least the agencies which are responsible for the retention of the sorbate are more effective.

The experiments on rate of sorption gave an indication of the time required to establish equilibrium when points on the sorption isotherm were being obtained. When the relative vapor pressure was below 60% two hours was found to be ample. At higher relative pressures longer times were required, while at the saturation pressure 24 hr. was allowed to elapse.

4. *Effect of acid treatment of serpentine on sorption.* Since the sorptive properties of active silica are due to the removal of magnesium oxide and water from the parent mineral in such a way that the silica remains in the same form, the completeness of removal of the soluble constituents should exert a definite effect on sorption. In order to examine the optimum conditions of treatment, a number of complete isotherms have been obtained using silica that had been produced by different acid treatments. Points taken from these curves are listed in Table VI, together with details of the treatment accorded in each case.

TABLE VI

EFFECT OF ACID TREATMENT ON SORPTION

Sample No.	Acid	Conc.	Time of boiling, hr.	% Recovery	p/p_s			
					20%	50%	70%	95%
					x/m	x/m	x/m	x/m
23	H_2SO_4	1 : 3	3	47.8	5.8	10.5	13.2	—
32	H_2SO_4	1 : 3	6	44.5	6.1	11.0	15.4	22.5
49	H_2SO_4	1 : 3	16	42.7	5.6	10.0	14.2	19.2
39	H_2SO_4	1 : 3.5	3	46.3	5.4	10.0	13.8	19.0
55	H_2SO_4	1 : 2.75	3	45.6	4.5	9.0	12.6	18.2
66	HCl	20%	2	47.8	5.9	10.5	15.8	22.7
72	HCl	20%	4	46.2	5.0	10.2	14.5	20.8
86	HNO_3	35%	2	51.7	4.5	8.8	12.0	16.8
87	HNO_3	40%	3*	57.3	4.0	8.0	10.7	14.0

*60-90° C.

The empirical formula for serpentine is $3\ MgO.2\ SiO_2.2H_2O$. On the basis of molecular weight, and neglecting impurities such as iron, etc., complete removal of the magnesium oxide and the water of crystallization would

result in a recovery of 43.3% of the original weight of the mineral. Hence it will be seen that the results of the sulphuric acid treatments described in Table VI have approached this theoretical limit and in one case have actually passed it. The sorptive power seems to follow the degree of removal of soluble constituents. One exception to this appears in the table in the case of Sample 49, which was given the most vigorous treatment of all. There appears to be no appreciable difference between the action of hydrochloric acid and that of sulphuric acid, but nitric acid is definitely less effective, probably because the mineral is less soluble in this acid.

5. *Discussion of "dry" weight.* Active silica, like silica gel and many other sorbents, retains a certain amount of water in the so-called "dry" or "activated" condition. This water is associated with the solid in such a manner that it cannot be removed without resorting to drastic methods of heating which, if carried out, may permanently impair the sorbent. These conditions are not generally imposed and the "dry" point or "activated" condition, on which all sorption values are based, is an arbitrary value the magnitude of which is dependent on the conditions that are chosen.

In the experiments which have been described the samples as received had been heated to 120° C. and stored in airtight bottles. They were placed in the sorption balance without further treatment and evacuated until a constant weight was reached. This point was found to be reproducible in any given experiment although, as seen in Table VII, as much as 9% of water remains if the weight at 1000° C. is taken as zero. Table VII also contains values for silica gel, which shows a similar behavior. The sorptive power is reduced by such treatment but, owing to its rigid structure, active silica is less affected by high temperature than is silica gel. Since any reproducible point will obviously serve as a "zero" it is unnecessary to resort to this drastic treatment in every case.

TABLE VII*

"DRY" WEIGHT OF ACTIVE SILICA AND SILICA GEL

Temp., °C	% Water			
	Active silica			Silica gel
	1	2	3	
120	9.2	8.1	7.8	7.4
360	6.9	7.2	5.8	4.7
600	3.3	2.1	2.9	2.0
700	1.2	0.4	0.7	0.6
850	0.2	0.4	0.3	0.1
1000	0.0	0.0	0.0	0.0

*Supplied by D. Wolochow.
NOTE: *Weight at 1000° C. taken as zero.*

Sorption of Organic Vapors

The sorption of benzene and ethyl alcohol vapors has been examined in a manner similar to that described for water.

Tables VIII and IX contain typical results of these experiments. The magnitude of sorption is noticeably less than in the case of water, though the isotherms present a number of common features (see Fig. 6).

TABLE VIII

SORPTION OF BENZENE VAPOR BY ACTIVE SILICA AT 23° C.

Relative vapor pressure, %	x/m . 100	x/m . 100
Sorption		
11.9	5.5	5.9
25.2	8.0	8.9
32.8	8.4	9.4
65.0	12.9	13.5
74.0	13.9	15.1
100.0	16.3	16.7
Desorption		
83.5	15.5	15.5
19.9	7.2	7.5
6.1	4.4	4.4

TABLE IX

SORPTION OF ETHYL ALCOHOL VAPOR BY ACTIVE SILICA AT 23° C.

Relative vapor pressure, %	x/m . 100	x/m . 100
Sorption		
6.5	3.2	3.7
33.8	6.1	5.7
54.9	8.3	8.2
79.3	12.2	11.8
100.0	15.5	15.8
Desorption		
83.3	13.3	13.3
61.5	10.0	10.1
48.8	7.8	7.8
30.7	5.9	5.8
16.0	4.6	4.3

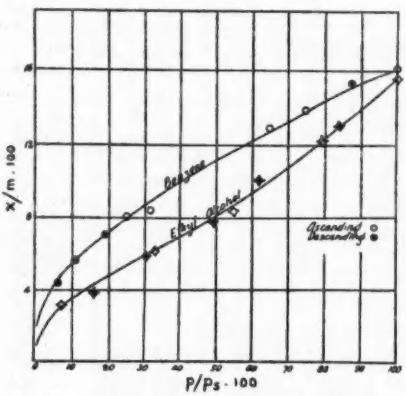

FIG. 6. *Sorption of benzene and alcohol by active silica at 23° C.*

No hysteresis was observed during the sorption of benzene or ethyl alcohol by active silica, the "ascending" or "descending" points falling on the same curve. The shape of the alcohol isotherm is distinctly reminiscent of the water curve showing the same sigmoid shape, resulting in relatively higher values for sorption near the saturation point. The curve for benzene, on the other hand, is rather like the type of curve obtained with silica gel. These differences are emphasized when the isotherms are plotted according to various sorption equations (see Discussion).

In Tables VIII and IX the values have been listed for the two samples which were examined concurrently in this particular experiment. Complete values have been given to bring out a very interesting fact which was frequently noticeable during the sorption of these organic vapors. In Table IX it will be seen that much closer agreement exists between the two samples during desorption than during sorption. The values in the former case are almost invariably identical while, during sorption, variations as large as 1.2% are evident. That these variations cannot be due to experimental error is shown by the accuracy of the desorption points. Hence they must be connected with the sorption process itself. This fact may have a bearing on the theory of sorption. It seems that in order to explain the process of sorption some "condensation" of the sorbed phase must be postulated, employing this term in its widest sense. It appears that as the molecules are removed

from this condensed state, more regular and uniform conditions follow than when they assume it. That is, values of sorption obtained on a "descending" curve are likely to be more uniform and repeatable than those obtained on an ascending curve.

II. Dynamic Method

Though the sorption isotherm is the ultimate indication of the sorptive power of any substance, it fails to give certain information which is desirable for the practical utilization of the sorbent. This is because it considers only final equilibrium values and in practice these are seldom attained, as a sorbent which is completely saturated at any relative vapor pressure has, in effect, ceased to be a sorbent. Hence for technical purposes, sorbents are frequently compared with regard to their efficiency, that is, their power to remove a sorbate from a stream of air or other indifferent gas. When the removal is complete and the exit gases show no trace of the sorbate the efficiency is 100%. When this condition no longer holds and vapor appears in the outlet gas, the sorbent has reached the "break point"; it will, of course, continue to take up vapor until the saturation value corresponding to the particular partial pressure and temperature is reached, but in practice it is not desirable to exceed the "break point," as an appreciable loss of vapor will occur.

Efficiency may be measured by ascertaining the time during which the removal of vapor by a given amount of sorbent under definite conditions is complete. The dynamic sorption apparatus illustrated in Fig. 7 was employed. The saturator consisted of a triple bubbler fitted with fluted filter paper to increase the liquid surface. The vapor pressure was adjusted by placing the saturator in a separate thermostat. Suitable spray traps were interposed. The vapor-air mixture passed from these through a copper coil immersed in the primary thermostat to ensure that the mixture entered the sorption tube at the same temperature as the sorbent.

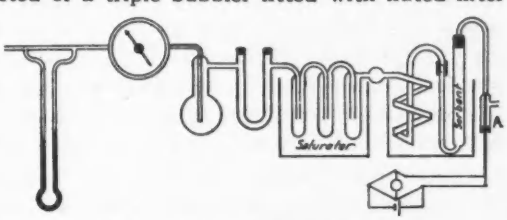

Fig. 7. *Dynamic sorption apparatus.*

The experiments were carried out using water, as the sorbate, the presence of moisture in the air leaving the sorbent being detected by a device described by Anderson (1) and shown at *A*, Fig. 7.

A small globule of calcium chloride is fused between two copper wires placed 1 mm. apart. The wires are placed as the unknown in a Wheatstone bridge circuit. The device is arranged so that the air to be tested must pass over the calcium chloride. With dry air the resistance of the circuit was about 100,000 ohms, while a trace of moisture reduced this value to about 500 ohms. An a-c. bridge circuit gave the most satisfactory results. It was unnecessary to fuse the globule between successive determinations as it

could be readily dried by passing dry air through the tube. When dry air passing through the apparatus was replaced by air at a relative humidity of 25%, the detector responded actively to the change in approximately five seconds.

The sorption tube containing some 4 gm. of the sample to be examined was placed in an oven at 155° C., and a gentle stream of dry air was passed through the tube for two hours. After the tube was weighed it was placed in the sorption train and moist air was drawn through at a known partial pressure until the presence of water vapor in the outgoing gas indicated that the sorbent had ceased to operate at 100% efficiency. The time, amount of gas passed, and the weight of the sample were noted. A typical set of results are to be found in Table X.

TABLE X

EFFICIENCY OF ACTIVE SILICA AT 23°C.

Rel. hum., %	Vol. of air, litres	Time, min.	m, gm.	x, gm.	x/m . 100
96.0	31.91	38	4.226	0.5110	12.1
96.0	34.4	42	4.2170	0.4975	12.1
96.0	29.8	37	4.2230	0.4930	11.7
26.3	100.0	120	4.2230	0.3410	8.1
26.3	98.3	112	4.2210	0.3240	7.8

The effectiveness of this sorbent may be seen from the fact that its volume was approximately 5 cc. which, with an average gas velocity of 830 cc. per min., resulted in a time of contact between gas and sorbent of approximately 0.3 sec., yet the removal of water vapor was complete for the times shown in Table X. After the appearance of moisture at the outlet the silica continues to take up water until the composition of the outlet gas is the same as that of the entering gas. This point will be the equilibrium point for the particular vapor pressure and temperature and will correspond to that appearing in the isotherm given above, subject, of course, to correction for the different manner in which the "dry point" was obtained.

Comparison of active silica with other common sorbents. Table XI shows a comparison of the saturation values for active silica with those of some other more well known substances commonly used as sorbents. The values for silica gel were obtained in this laboratory with the sorption balance, and refer to the commercial product. Values for activated alumina have been taken from the literature on the subject. Other values for alumina are available, some showing higher values than those listed in Table XI. The saturation value in the case of water is corroborated in a pamphlet published by the makers of the commercial product.*

TABLE XI

SORPTION SATURATION VALUES FOR VARIOUS SUBSTANCES—SATURATION VALUES AT 25° C.; % BY WEIGHT

Sorbate	Alumina (10)	Active silica	Silica gel
Water	23.0	25.0	33.0
Benzene	15.7	17.2	25.0
Ethyl alcohol	12.9	15.2	19.1

*The Aluminium Company of America. *Properties of activated alumina.* 1930.

The same order as that appearing in Table XI is shown when the relative afficiencies are considered. Silica gel has been tested as described above end it was found that when saturated air passed through the gel at a rate of 100 cc. per min. per gm. of gel, the removal of water vapor was complete for 108 min., during which the gel took up 21% of its weight of water vapor. This value checks well with that given by Miller (9), who employed a different method of moisture detection.

Active silica at 100 cc. per min. per gm of gel remained 100% efficient for 76 min. and at the end of this time had taken up 12.1% of its dry weight.

Similar values for alumina were not measured but the makers of the commercial product* prescribe a rate of 10 cu. ft. per hr. per lb. of alumina, under which circumstances the gel will sorb water at 100% efficiency until water is taken up to the extent of 8 to 10% of the dry weight of the sorbent. This rate is much lower than that stated above, but the amount of sorption, when water vapor first appears in the outlet gas, is in accord with the values as obtained from static experiments and indicates that commercial alumina is somewhat inferior to active silica as a water absorbent.

In general it seems that this new sorbent should prove useful in technical processes, as its sorptive power compares favorably with other sorbents in common use. It may be readily reactivated and appears to maintain its desirable properties even when heated to relatively high temperatures. As it is produced from a natural product by a simple chemical reaction, its manufacturing cost should be less than that of most of the sorbents which have been exploited up to the present.

Discussion

The method of production of active silica consists in removing the acid-soluble components of a hydrated magnesium silicate in such a manner that the silica remains in the same crystal arrangement as before, or at least the mineral retains its original shape.

It is of interest to note that while this sorbent is obtained from a natural product, a somewhat analogous compound has been prepared in the laboratory. Holmes (5) describes a sorbent made by precipitating silicate of soda with ferric chloride under such conditions that ferric hydroxide is deposited in the pores of the silicic acid gel. "The novel feature of this process is the removal of ferric hydroxide by acid *after* the gel has been dried to a rigid non-collapsing structure. The traditional silica gel obtained capillarity by loss of water molecules."

It is at once apparent that this process is analogous to the production of active silica in which the "rigid non-collapsing structure" is the mineral serpentine. The product produced by Holmes has been called "chalky gel" to differentiate it from the hard glassy silica gel. Though their methods of production are somewhat different, both the physical properties and type of sorption of the "chalky" gels are very similar to those of active silica. They

* *The Aluminium Company of America. Properties of activated alumina. 1930.*

are both opaque, chalk-like substances, rather easily broken and quite different from silica gel which shows the hard, horn-like appearance which characterizes a dehydrated non-swelling gel.

Holmes (6, 3) showed that very high values of sorption could be attained by his gel at the saturation point of water and other vapors, values which were greatly in excess of those found in the case of silica gel, but owing to the shape of the isotherm, they were maintained only in the vicinity of the saturation point, and at lower relative vapor pressures vitreous silica gel showed higher sorption. The isotherm for vitreous silica gel tends to approach a definite saturation value after which no more sorption takes place. The curves obtained with Holmes' gel, on the other hand, show the same shape as those presented for active silica, so that relatively high values for sorption may be reached as the saturation pressure of the vapor in question is approached. (The actual values for "chalky" gels are much higher than for active silica). Owing to the shape of the curve in this region $d(x/m)/dp$ is changing so rapidly with pressure that the sorption value at the saturation point is indefinite.

Reference to the curves for active silica and water will show that the isotherms possess the characteristic shape of those of the "chalky" gel type. It appears therefore that if porosity is produced by the removal of a metal constituent from a silica mixture, the resulting sorption isotherm is such that very high values are attained as the saturation pressure is approached. In the case of the "vitreous" silicic acid gel, porosity is produced by the removal of water molecules, and the isotherm tends to show a definite saturation at some pressure below the saturation pressure of the liquid.

These two types of isotherms have been referred to previously, and it is of interest to note that in this case they appear with the same substance, depending on the manner in which it was prepared. The shape of the isotherm may also depend on the vapor, as it has been shown that benzene gives the C-shaped curve, while water gives the sigmoid curve with active silica. Munro and Johnson (11) have found similar results in the case of sorption by alumina. They point out that "when the vapor pressure is two-thirds of the saturation pressure, the gel has taken up only one-fifth of the saturation amount of water, whereas three-fourths of the total quantity of benzene is held at this relative pressure."

It is clear that no single sorption equation will fit these isotherms throughout their whole length. The ordinary empirical sorption formula, $x/m = ap^{\frac{1}{n}}$ is applicable in certain cases, if the lower portion only of the isotherm is considered. In isotherms of the type shown by the benzene-silica gel system, it fails completely, the logarithmic plot being a flat curve concave to the pressure axis. The flat curve changes to a horizontal line at higher relative vapor pressures. The curves obtained with active silica, on the other hand, show some agreement with the formula at relative vapor pressures less than 60%, while the silica gel-water system shows agreement up to 50% relative vapor pressure. These curves are illustrated in Fig. 8.

McBain (8) has shown that in the case of charcoal, good agreement with the observed results is obtained by application of the Langmuir formulation, $x/m = \frac{abp}{1 + ap}$. The isotherms for silica gel and benzene are similar to the charcoal type in that most of the sorption takes place before half the saturation pressure is reached. The various isotherms for silica gel have been plotted according to this formula in Fig. 9, the values for silica gel having been taken from a previous paper (12). It will be seen that agreement exists in the case of the alcohol and benzene systems, though water shows no correlation. Active silica shows some agreement in the case of benzene, but of course the sigmoid isotherm appearing in the water and alcohol systems cannot be represented by the Langmuir equation.

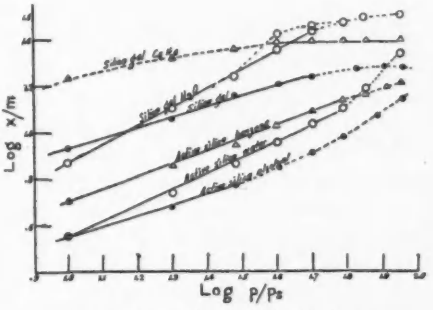

FIG. 8. *Logarithmic plot of isotherms.*

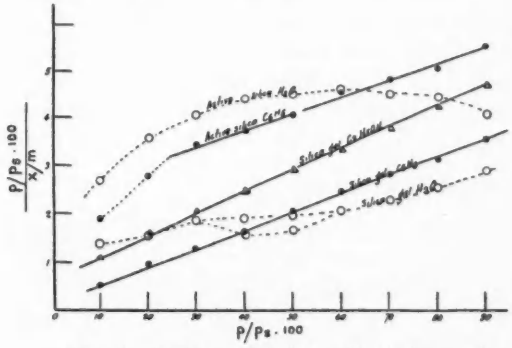

FIG. 9. *Isotherms plotted according to Langmuir equation.*

McBain has amply demonstrated in the case of charcoal that the concept of capillary condensation may not be applied to systems where most of the sorption takes place at low relative vapor pressures. It seems that his argument may be logically extended to the silica gel-benzene system, so that sorption in this case may be considered as a direct surface phenomenon which ceases when the available surface has become saturated. The chalky gels, however, show the characteristic increase in sorption near the saturation point. This suggests that condensation of liquid is taking place in this region at least.

In general the amount of sorption which takes place at any temperature is governed by the natures of the gas and the solid, the partial pressure of the sorbate, and the characteristic structure of the solid. The systems under discussion present a case in which the fundamental natures of the solids are the same, yet the type of sorption is quite different at higher partial pressures. Condensation of liquid appears to be possible with the "chalky" gels but not with "vitreous" gels, as in the latter case sorption rises to a maximum before the saturation pressure is reached. If both solids are assumed to be of the

same nature, the differences in behavior must be due to a different gel structure. It is generally assumed that the sigmoid isotherm is produced by a sorbent of larger pore diameter.

In this connection it is interesting to note that Freundlich (4) has suggested that differences in the shape of isotherms near the saturation pressure may be due to the wetting power of the condensed phase for the solid. When the condensed phase readily wets the solid, liquid may appear and the amount of sorption rises very sharply, reaching high values at the saturation point. On the other hand, if the liquid does not wet the sorbent, or does so with the formation of an angle of contact, then the vapor behaves as a gas above its critical temperature, and no increase in sorption takes place and the isotherm does not rise near the saturation point. It should follow that an isotherm of the "wetting" type would change to the "non-wetting" type above the critical temperature. Very little work of this nature has been carried out owing to the experimental difficulties which are involved and, in such experiments as have been carried out, sorbents showing the "non-wetting" type at ordinary pressures have been employed.

The actual nature of the pore walls may bring about this difference, as shown by Bonnell (2) in the case of silica gel. He found that the undialyzed gel produced the isotherm giving high sorption values at the saturation point, though the dialyzed gel gave the ordinary isotherm showing hysteresis. These results were obtained even when the gels were dried; hence the acid remaining on the pore walls brought about the significant change in the type of sorption.

Acknowledgment

The author wishes to acknowledge his indebtedness to Mr. D. Wolochow, of the Division of Chemistry, National Research Laboratories, who developed the method of preparation of the active silica and who prepared all the samples; and to Dr. G. S. Whitby, Director of the Division, at whose suggestion this investigation was carried out.

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THE MOLECULAR DIAMETER OF DEUTERIUM AS DETERMINED BY VISCOSITY MEASUREMENTS[1]

By A. B. Van Cleave[2] and O. Maass[3]

Abstract

The viscosities of deuterium over the range 23 to −183°C. have been measured. The viscosities of the two-component system deuterium-hydrogen have been measured over the whole concentration range at 22°C. The results show that the deuterium molecule has the same diameter as the hydrogen molecule. The interest attached to the viscosity results for the two-component system are pointed out. The difference between the molecular volume of liquid deuterium oxide and that of liquid water is attributed by the authors to a difference in equilibrium between associated and non-associated molecules in the respective systems.

Experimental

The apparatus used in the viscosity determinations was essentially the oscillating disc type described in detail by Sutherland and Maass (2). A few minor improvements have been made. These will be described in a subsequent paper. This apparatus is ideally suited to the measurement of viscosity coefficients where only small amounts of gas are available. Besides, it has the advantage of affording an exceedingly accurate method of measuring gaseous viscosities at all temperatures below room temperature. It is estimated that the values given below are correct to better than 0.20%, and have a relative accuracy of 0.10%.

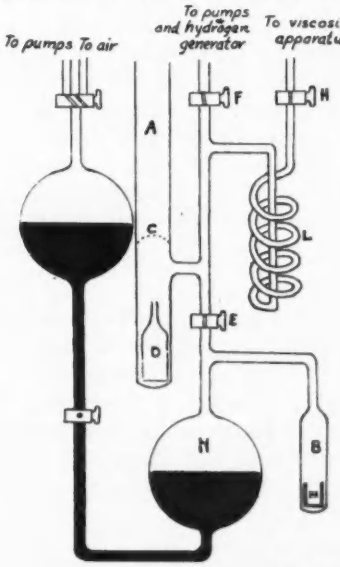

Fig. 1. *Diagram of apparatus used in preparation and purification of deuterium.*

Preparation and Purification of Deuterium

The deuterium was prepared from one gram of deuterium oxide (sp. gr. 1.1058), which was purchased from the Ohio Chemical and Manufacturing Company. The deuterium was liberated by allowing the oxide to react with pure metallic sodium. Fig. 1 is a diagram of the apparatus used in the preparation and purification of the deuterium.

The apparatus was first evacuated and filled with dry air. The tip was then cracked off the deuterium oxide container, *D*, which was quickly lowered into the tube *A*. *A* was then immediately sealed off at *C*. By making the tube *A* long, the sealing off could be accomplished without causing condensation of any

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[3] *Macdonald Professor of Physical Chemistry, McGill University.*

water, from the flame, on the inside of the tube. The deuterium oxide was then frozen in liquid air and the whole system evacuated and flushed with pure dry hydrogen. The frozen deuterium oxide was allowed to melt in the presence of the hydrogen to expel dissolved air. Tube *A* was again immersed in liquid air and the system thoroughly evacuated. Tube *B* containing the aluminium cup *M*, nearly filled with metallic sodium, was then surrounded by liquid air while *A* was allowed to warm up to room temperature. When all the deuterium oxide had sublimed into *B* the system was again evacuated and the stopcock *E* closed. The liquid air was then removed from *B*, allowing the reaction to take place.

The deuterium was collected over very pure mercury in the 500 cc. bulb *N*. When *N* was nearly filled with gas the reaction was stopped by replacing the liquid air around *B*. Stopcock *E* was then opened, *H* and *F* being closed. The coil *L* was surrounded by a Dewar flask containing liquid air. Stopcock *H* was then partly opened and the deuterium slowly forced into the evacuated viscosity apparatus. Previous to evacuation the viscosity apparatus had been flushed with pure, dry hydrogen. The reaction in *B* was then allowed to proceed, being aided by heating to 200°C. In this manner enough deuterium was obtained to fill the viscosity apparatus to a pressure slightly greater than atmospheric at room temperature. The gas pressure in the viscosity apparatus could be read on an attached absolute manometer.

The method of making the deuterium-hydrogen mixtures was as follows. Part of the original deuterium was removed from the viscosity apparatus and the pressure noted. Very pure hydrogen was then admitted and the pressure again read, the temperature being maintained constant during this operation. The molecular percentages of the constituents were calculated directly from their partial pressures. All mixtures were allowed to stand for 24 hr. before making any viscosity determinations, thus insuring perfect mixing of the two gases.

Results

Before making any measurements on deuterium, check determinations were made on pure hydrogen prepared by two different methods:— (a) hydrogen from a Kipp generator, purified as indicated by Sutherland and Maass (2); and (b) hydrogen prepared by allowing pure distilled water to react with metallic sodium, as outlined above. In all cases the values for the viscosity coefficients agreed with those given by Sutherland and Maass (2) within 0.16%. This affords an excellent check on the purity of hydrogen prepared by the sodium method.

Since the specific gravity of the original deuterium oxide was 1.1058 it contained 98.0% of D_2O by weight. Hence, on a basis of molecular percentages the deuterium formed 96.08% of the mixture, the remainder being ordinary hydrogen.

Table I is a summary of the results obtained with this mixture. Each value is the mean of three or four independent runs. These results are shown graphically in Fig. 2.

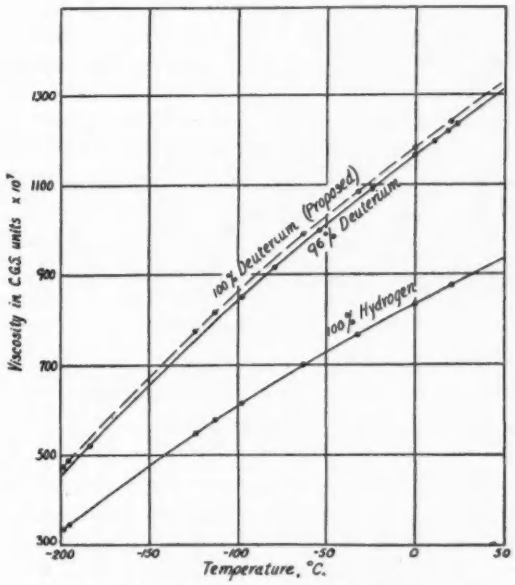

FIG. 2. *Variation of viscosity with the temperature.*

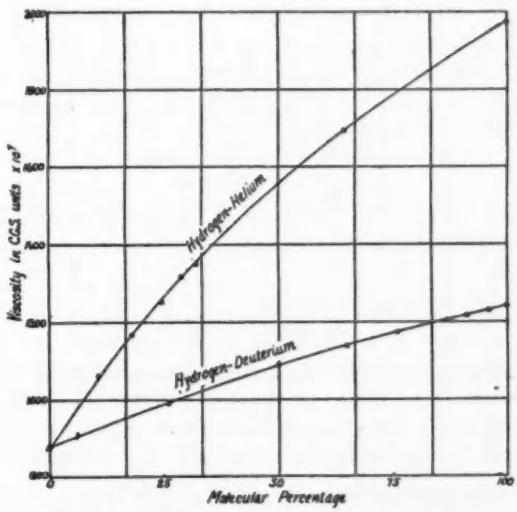

FIG. 3. *Variation of viscosity with concentration.*

TABLE I

VARIATION OF VISCOSITY WITH TEMPERATURE (98.0% BY WEIGHT DEUTERIUM, 2.0% BY WEIGHT HYDROGEN

Temp., °C.	$\eta\times10^7$	Temp., °C.	$\eta\times10^7$
23.61	1234.9	−24.23	1092.1
18.11	1218.9	−53.90	998.7
10.37	1196.3	−79.13	914.7
−0.19	1165.3	−183.00	520.0

Table II shows the result of diluting the deuterium with hydrogen. For comparison all the experimental values have been reduced to their values at 22.0°C. (fourth and eighth columns). As before, each value is the mean of at least three independent runs. The value for 100% deuterium was obtained by extrapolation. These results are graphically represented in Fig. 3.

TABLE II

VISCOSITIES OF DEUTERIUM-HYDROGEN MIXTURES

Deuterium, molecular per cent	Temp., °C.	$\eta\times10^7$	$\eta_{22}°\times10^7$ (calcd.)	Deuterium, molecular per cent	Temp., °C.	$\eta\times10^7$	$\eta_{22}°\times10^7$ (calcd.)
1.0000	22.00	—	1242.5	.6513	22.06	1138.3	1138.2
.9608	22.47	1232.4	1231.0	.4998	21.96	1088.2	1088.3
.9111	22.00	1217.0	1217.0	.2564	22.02	991.3	991.3
.8677	22.07	1205.1	1205.0	.0000	22.00	878.8	878.8
.7634	21.73	1173.6	1174.3				

Discussion

The well known expression

$$\eta = 1/3mnxl$$

can be reduced to $\eta = kM/\sigma^2$ for purposes of this discussion. η is the coefficient of viscosity of a gas, m the mass of one molecule, n the number of molecules in 1 cc. of gas, x the average velocity of the molecules, l the mean free path, M the molecular weight, σ the molecular cross section, and k a constant for a given temperature. Hence, we have

$$\frac{\eta_D}{\eta_H} = \sqrt{\frac{M_D}{M_H}} \cdot \frac{\sigma_H^2}{\sigma_D^2} ,$$

where the D subscripts refer to deuterium and the H subscripts to hydrogen. If $\sigma_D = \sigma_H$ then

$$\eta_D = \sqrt{\frac{M_D}{M_H}} \cdot \eta_H = \sqrt{2} \cdot \eta_H .$$

The extrapolation of the deuterium-hydrogen curve in Fig. 3 shows that the viscosity of deuterium at 22°C. is $1.414 \pm .002$ times the viscosity of hydrogen at that temperature. Thus, within the writers' experimental error the radii of deuterium and hydrogen molecules at 22°C. are the same.

The ratio $\eta_D : \eta_H$ was found to be constant over a large temperature range. and hence the molecular volumes of deuterium and hydrogen are the same.

In this connection it is of interest that the molecular volumes of liquefied D_2O and H_2O are not the same.

Lewis and MacDonald (1) give the specific gravity of pure D_2O at 25°C. as 1.1056, as against 1.111 calculated by assuming that D_2O has the same molecular volume as ordinary water. This means that the apparent molecular volume of liquid D_2O is greater than that of liquid H_2O.

If this were due to the difference between the volume of the deuterium atom and that of the hydrogen atom then, since the oxygen contributes a large part of the volume in the oxides, the difference would have to be greater than 1%. The viscosity experiments show that as far as the deuterium and hydrogen molecules are concerned a difference of such magnitude does not exist. To account for the difference between the molecular volume of liquid D_2O and that of liquid H_2O, the authors advance the hypothesis that the D_2O and H_2O molecules have the same volume but that in the equilibria

$$3\ D_2O \rightleftharpoons (D_2O)_3 \quad (1)$$

$$3\ H_2O \rightleftharpoons (H_2O)_3 \quad (2)$$

the first is displaced more in the direction of association than the second. In that case the difference in molecular volume ought to become less with rise in temperature, as this favors the formation of the unassociated molecules. Lewis and MacDonald found this to be the case.

Sutherland's equation fails to represent the variation of viscosity with temperature for most gases at low temperatures. The results of Sutherland and Maass (2) for hydrogen and the authors' results for deuterium do not conform to this equation. The relation proposed by Jean's, $\eta/\eta_o = \left(\frac{T}{T_o}\right)^n$, gives the best representation of the viscosity variation of hydrogen and deuterium over the temperature range of 25°C. to −80°C. With $n = 0.699$ the calculated values for the viscosity of 98.0 weight per cent deuterium check exceedingly well with the experimental values over this temperature range. However, at −183°C. the deviation of calculated from experimental value is 3.13%. Similar results are obtained when this equation is applied to pure hydrogen.

The whole question of temperature variation of viscosity at low temperatures is an interesting one, and the data accumulated in this laboratory will be published shortly.

From the curves in Fig. 2 it is found that over the temperature range 25° to −80°C. the 96 molecular per cent deuterium curve can be obtained from the 100% hydrogen curve by multiplying values on the latter by $1.395 \pm .003$. Below −80°C. this relation begins to fail until at −183°C. the 96 molecular per cent deuterium has a viscosity of only $1.368 \pm .003$ times that of hydrogen.

The proposed viscosity curve (Fig. 2) for 100% deuterium was constructed by extrapolation. From 25°C. to −80°C. this curve should be very nearly

the correct one. Deviations from the true values will be greater at the lower temperatures. Table III gives values taken from this curve.

The viscosities of gas mixtures are of considerable interest from the point of view of the kinetic theory. Deuterium-hydrogen and deuterium-helium mixtures are of particular importance. Now that it has been shown that the cross section of the deuterium molecule is the same as that of the hydrogen molecule, the approximations of the mean diameters necessary in some theoretical deductions are unnecessary. The first system therefore is one in which molecules of equal diameter but different weight influence the viscosities. In the second system the molecules are of equal weight but have different molecular diameters. The data for the first system are shown graphically in Fig. 3. In this figure the data for the hydrogen-helium system, as determined by Trautz and coworkers (3, 4), are given for purposes of comparison. The authors intend to obtain the data for the deuterium-helium system, and when this is done the relations deduced for mixtures by Trautz, Chapman and others will be critically examined. Preliminary calculations on the system deuterium-hydrogen seem to show that further amplification of the theory is necessary.

TABLE III

VISCOSITIES OF DEUTERIUM AT DIFFERENT TEMPERATURES

Temp., °C.	$\eta \times 10^7$	Temp., °C.	$\eta \times 10^7$
20	1240	−100	861
0	1181	−120	787
−20	1120	−140	710
−40	1060	−160	632
−60	997	−180	550
−80	931	−200	468

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VISCOSITY OF GLUTEN DISPERSED IN ALKALI, ACID AND NEUTRAL SOLVENTS[1]

By R. C. Rose[2] and W. H. Cook[3]

Abstract

The viscosity of gluten dispersed in urea and sodium salicylate was higher, and showed a greater increase with increasing protein concentration, than that of dispersions of the same age and concentration, in sodium hydroxide and acetic acid solutions. Calculations, based on these measurements, indicated that the effective particle size is larger in the former pair of solvents. In urea solutions the viscosity of dilute gluten dispersions was independent of the hydrogen ion concentration between pH 6.1 and 9.2, and within this range the system was stable. Beyond this pH range the viscosity at first increased, but the system was unstable as shown by a subsequent rapid decrease in viscosity with time.

Dilute dispersions in sodium hydroxide, urea and sodium salicylate solutions decreased in viscosity at first, whereas the viscosity of dispersions in acetic acid decreased continuously. Some evidence was obtained of coagulation in concentrated dispersions in the neutral solvents at 0° C. and 25° C.

The character of the precipitate obtained by salting out dispersions in each of the four solvents after storage at 25° C. indicated that the neutral solvents alter the gluten less than alkali or acid. This conclusion is supported by the fact that glutens obtained from flours of different protein quality had essentially the same viscosity when dispersed in alkali or acid, but in the neutral solvents exhibited markedly different viscosities which were partially correlated with the quality of the gluten.

1. Introduction

Viscosity has been used in this study as a measure both of the state of, and of the change in, dispersions of gluten in various solvents. It has been shown by Cook and Alsberg (7) and Cook and Rose (8) that wet gluten, obtained from wheat flour, can be dispersed completely in solutions of urea and sodium salicylate. The dispersing action of these solutions on coagulated proteins had already been demonstrated by other investigators (1, 11, 25) some of whom also showed that they denature, or otherwise alter, albumin and haemoglobin. In the present investigation of the viscosity of gluten dispersed in these, and in the classical solvents, dilute alkali and acid, an effort was made to determine the relative extent to which gluten was altered by dispersion in these four reagents.

A decrease in solubility is generally the first indication that a protein has been altered by a given treatment. The derivatives produced, namely, proteans, metaproteins and coagulated proteins, are defined, for the most part, by the nature of the treatment which produces them, rather than by any characteristic property of the resulting substance. It is probable that any difference in the solubility of these products represents a difference of degree rather than of kind.

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[3] *Biologist, National Research Laboratories, Ottawa.*

The coagulation of proteins has been studied more extensively than any of the other changes. Most of these investigations have been made on albumin or haemoglobin and, since these proteins can be crystallized, it appears to be reasonably certain that they are not altered in preparation. On the other hand, with other classes of proteins there is a possibility that the method of extraction and purification may alter the material to some extent before it can be studied systematically (15). Albumins can be coagulated in many different ways and it is not known whether the coagulation produced by these different methods is the same. The classical theory of coagulation is that it is the result of two distinct changes, denaturation and flocculation. The exact nature of the former is obscure but is believed to be chemical, while flocculation is regarded as a purely colloidal process which takes place under suitable conditions of hydrogen ion concentration and in the presence of certain electrolytes. Wu (26) and Wu and Chen (27) distinguish between the material produced by denaturation in acids and alkalis which flocculates at the isoelectric point, and the coagulum produced by the action of alcohol, heat, shaking, etc., in the isoelectric region. They (28) pointed out later that denaturation of certain proteins, including egg albumin, is accompanied by the liberation of non-protein substances, whereas coagulation is not. Wu regards denaturation as degradation, and coagulation as condensation. On the other hand, Sørensen and Sørensen (23) have shown that no nitrogen compounds of any kind are liberated from egg albumin during denaturation. In view of the conflicting evidence it seems probable that degradation is the result of secondary changes rather than of denaturation proper.

From the physical standpoint, the viscosity measurements of Anson and Mirsky (3) and Loughlin and Lewis (16) indicate that the process of denaturation results in some change in the state of aggregation even when flocculation is absent. Other work of Anson and Mirsky (2, 19) also indicates that denaturation of albumin and haemoglobin is not irreversible as it was once thought to be. The method which they employed for reversing denaturation suggests a physical rather than a chemical change. Hewitt (10), however, has questioned the method of denaturation employed by Anson and Mirsky and consequently their method of effecting reversal may be of little value in interpreting the nature of the changes involved. Further support for the view that denaturation is a colloidal phenomenon is given by the work of Bancroft and Rutzler (4).

The action of acid and alkali on proteins was discussed briefly in an earlier paper (7) and it is sufficient to mention here that the work of Sjögren and Svedberg (22) and Speakman and Hirst (24) has shown that the stability of such soluble proteins as albumins and of such insoluble proteins as keratin is restricted to a limited range of hydrogen ion concentration. It is also well known that gluten, like many other proteins, exhibits a maximum swelling and viscosity at hydrogen ion concentrations remote from the isoelectric point. The work of Sharp and Gortner (21), however, suggests that the high viscosity of gluten at pH 3.0 and pH 11.0 is an unstable transitory

condition, but it is not known whether in its final condition at these hydrogen ion concentrations the protein is denatured or otherwise degraded. The evidence presented by Bungenberg de Jong (6) also suggests that the properties of gluten are changed at hydrogen ion concentrations remote from its isoelectric range.

It is questionable whether the methods used in, and the results of, previous investigations of the denaturation of other proteins can serve to elucidate the changes which occur in the denaturation of gluten. It seems probable that the term 'denaturation' denotes different changes in different proteins, and consequently the changes which occur in gluten may be quite dissimilar to those which occur in albumin. In addition, native albumins are found in dispersion, whereas gluten occurs as a solid, and the dispersion of the latter in any solvent must change at least its state of aggregation. Furthermore, relatively strong solvents are required to disperse gluten and it is highly probable that these cause greater alterations in this protein than those which occur when albumins are dispersed in water.

As dispersed gluten can be treated quite drastically without evidence of precipitation, measurement of the amount of insoluble material, the usual criterion of alteration, cannot be used to study the changes resulting from treatments. Viscosity measurements, however, present attractive possibilities, since gluten dispersions, in common with other lyophilic colloids, are characterized by a high viscosity which is conditioned by the state of the dispersed phase.

Unfortunately it is difficult to relate the changes in viscosity to a definite alteration in the character of the dispersed material. Neglecting the electroviscous effect, the viscosity of a suspension, or of a true solution of large molecules, is given by the fundamental equation $\eta = \eta_0(1+k\varphi)$ where η_0 is the viscosity of the medium, k a constant, and φ the fraction of the volume occupied by the dispersed material. Empirical equations of higher degree have been developed by other investigators (13) to fit the results obtained with other systems, but all of these show that the viscosity is determined by the quantities given in the above formula. In these equations the constant k has been given different values (18) and as it is assumed to be dependent on the shape of the particles it has been termed a shape factor. Furthermore, for lyophilic colloids, the quantity φ is much larger than the product of the concentration c and the specific volume v of the dispersed phase and may therefore be termed the "equivalent hydrodynamic volume" (13). Similarly, the quotient φ/c is much larger than the specific volume and may be called the "specific hydrodynamic volume". The larger volume may be explained by assuming that the dispersed particles are either greatly hydrated, or interlocked and connected to form a micellar structure (13, 18). Although true hydration is undoubtedly a factor in determining the swelling and aggregation, and consequently the effective size of the dispersed gluten particles, it seems probable that the high viscosity can be attributed mainly to the mechanical immobilization of the dispersion medium by the presence of bulky

aggregates, or by elongated particles that increase the resistance to shear through mutual entanglement.

The electro-viscous effect adds still another complicating factor. Its magnitude is unknown owing to the existing uncertainties as to the values of the effective dielectric constants and the absolute magnitudes of the electrokinetic potentials in the systems under investigation. It is probable, however, that the electro-viscous effect in the neutral solvents is small compared with the effect of the state of aggregation.

It follows from the above reasoning that a change in the shape or size of the particles may alter the viscosity. From an experimental standpoint Freundlich and Ishizaka (9) have shown that the coagulation of aluminium hydroxide sols is accompanied by an increase in viscosity, and have used viscosity determinations to measure the rate of coagulation. Anson and Mirsky (3) and Loughlin and Lewis (16), however, found that the denaturation of egg albumin was accompanied by an increase in viscosity regardless of whether aggregates formed or not.

In spite of the obvious importance of the problem no adequate investigations of solvents, designed to determine which cause the least change in gluten, have been made. It was hoped that in the present study alterations in viscosity could be taken as the chief criterion of change.

2. Methods

Preparation of Dispersions

The flour from which the gluten for the main series of experiments was obtained was milled from a high grade sample of hard red spring wheat (Marquis) and was free from bleaching agents and chemical improvers. In preparing the gluten, 250 gm. of flour was mixed into a firm dough and allowed to stand for 1 hr. in about four times its weight of distilled water, after which most of the starch was removed by kneading under a stream of tap water for 30 min. and finally under distilled water for 10 min.

The dispersing agents originally employed were 0.1 *N* sodium hydroxide, 0.1 *N* acetic acid, 30% urea and 12% sodium salicylate, but supplementary experiments were conducted with other concentrations of the neutral solvents. The authors have reported elsewhere (8) that 24% urea and 8% sodium salicylate are capable of dispersing gluten completely, but the work was begun with the higher concentrations mentioned above because reliable information regarding the minimum concentrations required was not then available. The concentrations of alkali and acid were chosen, after preliminary trials, as giving rates of dispersion within the range limited by the two neutral solvents.

Dispersion was accomplished by placing the wet gluten in the different solvents at 25° C. and shaking vigorously at frequent intervals, as it was found impossible to obtain complete dispersion in urea solution at 0° C. The wet gluten contained about 67% of water and the required amount of solid urea or sodium salicylate was added to obtain the required concentration

of the dispersing agent. No allowance for the water content of the wet gluten was considered necessary with the dilute solutions of acid and alkali employed. As urea solutions tend to become alkaline on standing, these were stabilized at pH 6.8 using Sørensen's phosphate buffers ($KH_2PO_4+Na_2HPO_4$).

The average length of time required to obtain complete dispersion in the various solvents was: in sodium hydroxide, 2.5 hr.; in acetic acid, 1.5 hr.; in urea, 1.0 hr.; and in sodium salicylate, 3.0 hr. All dispersions were passed through a Sharples supercentrifuge at a rate of about 50 ml. per min, in order to remove the starch. As sodium salicylate dispersed the gluten less rapidly than the other solvents, dispersions in this reagent were centrifuged when 4 hr. old, and those in the other solvents when 3 hr. old. In all cases the centrifuged material was starch-free and the loss of protein in the centrifuge was slight.

As dispersions of a definite gluten concentration were required for most experiments, the amount of gluten added originally was slightly in excess of that required to give the desired concentration. After centrifuging, the dispersion was analyzed for protein nitrogen, and then diluted with the solvent to the required concentration.

Nitrogen Determination

The ordinary Kjeldahl method was used to determine the nitrogen in weighed samples of the dispersions in alkali, acid and sodium salicylate. With the urea solutions it was necessary first to precipitate the gluten and, trichloracetic acid failing to give quantitative results, complete precipitation was achieved satisfactorily by a freshly prepared solution containing 50 gm. of tannic acid and 25 ml. of concentrated sulphuric acid per litre (17). Forty ml. of this reagent was poured into a 100 ml. centrifuge tube together with enough distilled water to make the volume 100 ml., after addition of an accurately weighed quantity of gluten dispersion, containing approximately 50 mgm. of protein nitrogen. After stirring thoroughly, the mixture was centrifuged and the liquid decanted. The precipitate was washed three times by adding 5–7 ml. of the tannic acid reagent, triturating, diluting to about 100 ml. while stirring, and then centrifuging and decanting. After the third washing the precipitate was transferred to a Kjeldahl flask.

The method was tested by applying it to dispersions in which the weight of wet gluten and of urea solution were both accurately known and which were not passed through the supercentrifuge. The nitrogen content of the wet gluten having also been determined, it was possible to compare the quantities calculated from the weights of samples used with those found by the tannic acid precipitation method. The results given in Table I show that the method was satisfactory, the maximum difference between the value calculated and that found being 0.10 in 8.32 or 1.2%.

Since some of the dispersions were too viscous to be pipetted accurately, all portions taken for analysis and dilution were weighed and, in consequence, the gluten concentrations are expressed throughout this paper as mgm. of protein nitrogen per gm. of dispersion.

TABLE I

RECOVERY OF PROTEIN NITROGEN FROM GLUTEN DISPERSIONS IN 30% UREA

Nitrogen content of wet gluten		Weight of wet gluten, gm.	Weight of dispersion, gm.	Mgm. of protein N per gm.	
Replicates, %	Average, %			Calculated	Determined
4.27, 4.29, 4.27, 4.38	4.30	9.339	82.280	4.88	4.83
4.26, 4.18, 4.23, 4.44	4.28	6.003	31.934	8.05	8.07
4.32, 4.39, 4.35	4.35	3.931	20.551	8.32	8.22
4.66, 4.59, 4.70, 4.64	4.65	38.85	180.0	10.04	10.02
4.23, 4.26, 4.01, 4.12	4.15	5.361	16.579	13.42	13.32

Viscosity

As the viscosity of such lyophilic colloids as protein dispersions is, in general, dependent on the method of measurement, and particularly on the rate of shear, it is frequently referred to as an "apparent" rather than a "true" value. Comparable results can be obtained only in a carefully standardized type of viscometer in which the rate of shear is approximately the same for dispersions of different viscosity. Ideally this would require a different viscometer for each viscosity but, this being impracticable, the set of standard U-tube viscometers recommended by the British Engineering Standards Association (5) was employed. Although these viscometers are, for several reasons, not ideal for measuring the apparent viscosity of protein dispersions, they possess the advantage of providing a series of standard design, each of which is used over only a limited viscosity range. The results obtained, therefore, can justifiably be expressed in absolute rather than in relative units.

The viscosities of the dispersions studied fell within the ranges of the No. 1 and No. 2 viscometers of the set, namely, 0.9 to 7.2 and 5.4 to 43.0 centipoises respectively. The No. 1 viscometers were standardized at three points within their viscosity range, using water and 20 and 40% sucrose solutions at 25° C., and the No. 2 viscometers at four points, namely, 40% sucrose solution at 20 and 25° C., and 60% sucrose at 25 and 30° C. The sucrose used was chemically pure and had a melting point of 186° C. All temperatures were maintained within 0.02° C. of the stated values, and the densities of the liquids used were determined at the same temperatures using 25 ml. specific gravity bottles with drilled stoppers. In weighing, the usual corrections were made for the buoyancy of air, etc. The viscosity of the solutions used was obtained from the International Critical Tables (12). The results of the standardization showed that the constant applicable to a particular viscometer could be used throughout its entire range, as the variation in this quantity never exceeded 0.5%, and showed no evidence of being systematic.

A further check on the standardization of the viscometers was obtained from measurements made on the dispersing agents. The results obtained are given in Table II, together with the viscosities for these solutions as given in the International Critical Tables. There is good agreement between the two sets of values excepting for urea solutions, for which the determined values fall closer to a straight line than the corresponding values given in the Critical Tables.

TABLE II

VISCOSITY OF SOLVENTS

Solvent	Viscosity, centipoises	
	Determined	I.C.T.*
0.1 *N* acetic acid	0.90	—
0.1 *N* sodium hydroxide	0.92	0.92
24% urea	1.15	1.09
30% urea	1.21	1.16
36% urea	1.29	1.26
40% urea	1.34	1.33
30% buffered urea	1.22	—
8% sodium salicylate	1.10	1.09
10% sodium salicylate	1.17	1.16
12% sodium salicylate	1.22	1.23

* *Obtained by graphical interpolation where necessary.*

The viscosity and density measurements on the gluten dispersions were all made at a temperature of 25.0±0.02° C. Usually only two readings were taken, but if these did not check within 0.2% the determination was repeated.

Salting-out Tests

The method employed in salting out the dispersions in the neutral solvents was to add slowly a definite quantity of magnesium sulphate solution to 10.0 ml. of gluten dispersion. To effect measurable precipitation a 20% solution was required for salting out the gluten from urea solutions, and a 5% solution for dispersions in sodium salicylate. After adding the salt solution the dispersion was shaken, allowed to stand for one hour at room temperature, centrifuged in an ordinary centrifuge, and the precipitate washed with a solution of the same composition as that in which precipitation occurred. The nitrogen content of precipitates from dispersions in sodium salicylate was then determined directly. Precipitates from urea dispersions were redispersed in 30% urea solution and these analyzed for protein nitrogen, using the tannic acid method described above.

Hydrogen Ion Concentration

This determination was made at room temperature using a Clark hydrogen electrode and a calomel electrode containing a saturated potassium chloride solution.

3. Experiments

The first experiments performed were concerned with the reproducibility of the viscosity results, and the effect of gluten concentration on the viscosity of dispersions in all four solvents. Further experiments were then made on the effect of solvent and of hydrogen ion concentration on the viscosity of dispersions in the neutral solvents. The viscosity changes following dispersion were studied during storage at 0 and 25° C. in order to determine whether, and when, a constant viscosity level was reached in each of the reagents. The results of these experiments demonstrated that the viscosity of gluten dispersions, and the changes which occurred during storage, depended on the

solvent, but gave no evidence as to which of the solvents maintained the original properties of the gluten to the highest degree.

Two additional experiments were performed to obtain more definite evidence on this point. In the first of these, gluten dispersed in each of the four solvents was submitted to prolonged storage and then recovered, where possible, by salting out, and examined. In the second experiment, the viscosity of dispersions of gluten of different quality was studied with the object of determining whether or not the difference between the glutens was reflected in the viscosity of their dispersions. Solvents in which the different glutens have different viscosities, if these are related to the quality of the flour, can reasonably be regarded as superior to solvents in which all glutens have the same viscosity, since the latter condition suggests that certain of the original properties of the gluten have been lost.

TABLE III

REPRODUCIBILITY OF THE VISCOSITY OF DISPERSIONS CONTAINING 5 MGM. OF PROTEIN NITROGEN PER GM.

Solvent	Age of dispersion, days	Viscosity, centipoises
0.1 *N* sodium hydroxide	1	1.71
	1	1.92
	1	1.73
0.1 *N* acetic acid	3	1.70
	3	1.76
	3	1.78
30% buffered urea	1	4.12
	1	4.12
	1	4.03
10% sodium salicylate	1	3.47
	1	3.48

Reproducibility

In order to determine whether consistent results could be obtained, a number of gluten dispersions containing 5.00 mgm. of protein nitrogen per gm. of dispersion were prepared and their viscosity measured. These measurements, together with those from certain subsequent experiments, are given in Table III. The viscosity of the dispersions in acid and alkali exhibits the greatest variability. The maximum difference between the different dispersions in the neutral solvents is about 2%.

Effect of Gluten Concentration

In order to reach a decision as to the best concentration of protein to use in subsequent experiments, dispersions containing 4.30, 5.24, 8.00, 9.48 and 10.7 mgm. of protein nitrogen per gm. of dispersion were prepared in 30% urea solution. The viscosity of these dispersions when one day old, taken from the time of placing the wet gluten in the solvent, is plotted against the concentration in Fig. 1. Dispersions of protein concentrations higher than 10.0 mgm. of nitrogen per gm. were found somewhat difficult to handle, so this concentration was chosen as the maximum for use in subsequent experiments with all solvents and a concentration of 5.00 mgm. of protein nitrogen per gm. was used as representative of the more dilute dispersions.

The viscosity-concentration relation in the other solvents was not specifically investigated. However, incidental to the subsequent preparation

of dispersions in these reagents containing 5.00 and 10.0 mgm. of protein nitrogen per gm., a few results were collected on the effect of concentration on viscosity, and these have also been plotted in Fig. 1. It is evident that the viscosity-concentration relation in the neutral solvents is distinctly curvilinear, while in acid and alkali it is almost linear.

The non-linear relation obtained in urea solutions shows definitely that the equation $\eta = \eta_0(1+k\varphi)$ is not applicable to this system regardless of the value of φ. Kunitz's (14) equation was therefore applied in an expanded form which included the cubic term, and also in its complete form. The equivalent hydrodynamic volume φ and the specific hydrodynamic volume φ/c of one mgm. of protein nitrogen were then computed using both forms of the equation. Both gave essentially constant values for φ/c at different protein concentrations in a given dispersing agent, but those calculated by the cubic equation were larger than those computed by the complete equation. The complete equation was used since Kunitz has shown that it gives values of φ/c which are in agreement with those obtained by other methods. The values of φ and φ/c thus computed are given in Table IV. The curves in Fig. 1 were constructed by substituting the average values of φ/c in Kunitz's equation.

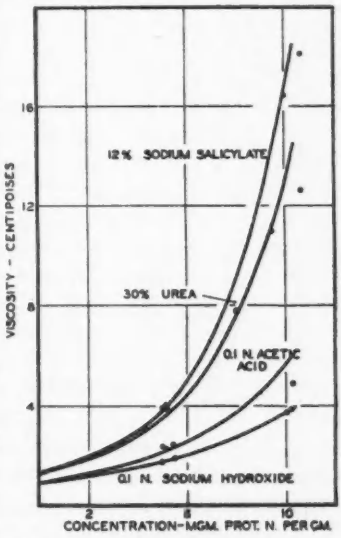

FIG. 1. *Effect of protein concentration on the viscosity of dispersions.*

TABLE IV

EQUIVALENT AND SPECIFIC HYDRODYNAMIC VOLUME OF DISPERSED GLUTEN

Solvent	Concentration, mgm. protein N per gm. (c)	Relative viscosity (η/η_0)	Equivalent hydrodynamic volume (φ)	Specific hydrodynamic volume (φ/c)
0.1 *N* sodium hydroxide	5.00	1.86	0.130	0.026
	5.46	2.00	0.145	0.027
	10.0	4.07	0.275	0.027
	10.2	4.20	0.280	0.027
0.1 *N* acetic acid	5.00	2.54	0.180	0.036
	5.17	2.49	0.175	0.034
	5.46	2.67	0.200	0.037
	10.3	5.40	0.320	0.031
30% urea	4.30	2.50	0.186	0.043
	5.24	3.20	0.232	0.044
	8.00	6.44	0.345	0.043
	9.48	9.09	0.395	0.042
	10.7	10.40	0.415	0.039
12% sodium salicylate	5.00	3.23	0.235	0.047
	5.08	3.29	0.238	0.047
	10.0	13.45	0.452	0.045
	10.7	14.85	0.463	0.043

Effect of Solvent Concentration

In order to study the effect of different concentrations of the solvent on the viscosity of a system having a fixed protein concentration, portions of a dispersion in 30% urea solution were adjusted to various urea concentrations and the same protein concentration, by adding water, 30% urea, or solid urea. All adjustments were made by weight. The final protein concentration was, in all cases, 4.30 mgm. of protein nitrogen per gm. After adjusting the urea concentration, the dispersions were stored at 0° C. for 12 hr. before the viscosity was determined. The results obtained are given in Fig. 2, together with the viscosity of aqueous urea solutions. It is evident that the increase in viscosity with increasing urea concentration is much larger in the presence of protein. This may possibly be explained hydrodynamically by increased mutual interference between the urea molecules and the large protein particles, but the system is too complicated to warrant further discussion.

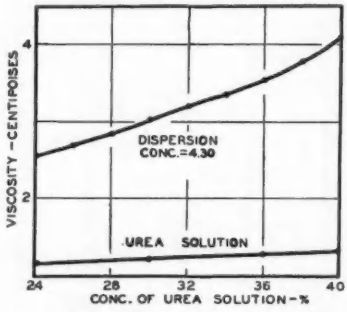

FIG. 2. *Effect of urea concentration on the viscosity of dispersions.*

No special study of the effect of the concentration of sodium salicylate on the viscosity of dispersions was undertaken, but during the course of the investigation, the viscosity of dispersions containing 5.00 mgm. of protein nitrogen per gm. in 8, 10 and 12% sodium salicylate was found to be 3.09, 3.47 and 3.94 centipoises respectively. This again shows the great effect of the concentration of the solvent on the viscosity of the dispersion.

Effect of Hydrogen Ion Concentration

An experiment was made to determine the effect of hydrogen ion concentration on the viscosity and stability of gluten dispersions in the neutral solvents. Portions of an 8-hr.-old dispersion in urea solution were adjusted to different hydrogen ion concentrations by adding buffer solutions, hydrochloric acid, or sodium hydroxide, made up in 30% urea. Sørensen's phosphate buffers were used from pH 6.12 to 8.05, but even within this range some dispersions were also adjusted with dilute acid or alkali in order to secure results comparable with those obtained outside this range. The final protein concentration of the dispersions was, in all cases, adjusted to 5.00 mgm. of protein nitrogen per gm. The hydrogen ion concentration of a 10-hr.-old dispersion in sodium salicylate was adjusted by adding solutions of sodium hydroxide in 10% sodium salicylate, again adjusting the final protein concentration to 5.00 mgm. of protein nitrogen per gm. No effort was made to prepare acidic samples in sodium salicylate owing to the insolubility of salicylic acid in water. All samples were stored at 0° C. after the hydrogen ion concentration was adjusted. As the dispersion medium had a different composition for each hydrogen ion concentration, the viscosity of

the media was determined. This varied from 1.20 to 1.21 centipoises in the urea solutions containing acid and alkali, and from 1.22 to 1.25 centipoises in those containing phosphate buffers. The viscosity of the sodium salicylate media varied from 1.17 to 1.19 centipoises. These small differences in the viscosity of the solvents would have little effect on the results.

It is evident from Fig. 3 that in urea solutions between pH 6.1 and 9.2 the viscosity is independent of the hydrogen ion concentration. On the acid side of this range the viscosity increases markedly with increasing acidity up to pH 3.9, the most acidic condition employed. On the alkaline side the viscosity increases with pH from pH 9.2 to 10.7 where it reaches a maximum and then decreases as the pH increases to 12.4, the most alkaline reaction studied. Moreover, it was found that between pH 3.9 and 6.1 and between pH 9.2 and 12.4 the viscosity decreased so rapidly with time that it was impossible to get consistent results. In determining the viscosity in these ranges, the first measurement was started five minutes after the sample was placed in the viscometer, since this was found to be sufficient time for it to reach 25° C. As each determination required about 10 min. the difference between the first and third reading corresponds to the viscosity decrease over a period of about 20 min. and is represented by the cross-hatched portion between the two curves in Fig. 3. It is evident from this figure that the viscosity fall-back is proportional to the viscosity increase. From the work of Sharp and Gortner (21) it seems highly probable that, had observations been made at pH values below 3.9, the viscosity would have been found to reach a maximum at about pH 3.0 on the acid side and doubtless the viscosity fall-back with time would have been found to be related to the absolute viscosity as in the alkaline region. It should also be noted that since the dispersions had doubtless suffered a viscosity decrease during the preceding storage at 0° C., and a further fall-back while being brought to a temperature of 25° C., even the upper curve by no means represents the maximum viscosity attained by the system.

The relative rate of fall-back at different hydrogen ion concentrations over a period of 24 hr. at 0° C. is shown in Table V by the difference between the observed viscosity of the dispersions when one day and two days old. Between pH 6.1 and 9.8 no significant difference was observed but beyond this range the viscosity decreased. The values given are those obtained from the first observation made on the two successive days. It is evident that there is a close correlation between the viscosity decrease at 0° C. and that observed at 25° C., although comparisons of the magnitude of the decrease

TABLE V

DECREASE IN VISCOSITY OF DISPERSIONS IN 30% UREA DURING STORAGE AT 0° C. AT HYDROGEN ION CONCENTRATIONS OUTSIDE THE STABLE RANGE

pH	Viscosity, centipoises		
	1 day old	2 days old	Decrease
3.88	7.73	7.30	0.43
4.40	6.44	6.09	0.35
5.65	4.89	4.77	0.12
10.2	4.88	4.76	0.12
10.6	5.49	5.18	0.31
11.6	4.21	4.16	0.05

observed over a 24-hr. period at 0° C., with those obtained over a 20-min. period at 25° C., show that the system is much more stable at the lower temperature.

The data plotted in Fig. 3 show that the viscosity of dispersions in 10% sodium salicylate is independent of the hydrogen ion concentration from pH 6.7, the most acid reaction studied, to pH 9.2. More alkaline dispersions increase slightly in viscosity as the pH increases to about 10.0 and then decrease again as the pH increases to 11.4, the most alkaline condition used. At pH values higher than 9.2 these dispersions appear to be more stable than those in urea, since no decrease in viscosity was observed over a 20-min. interval although a longer storage period at 25° C. might have revealed a behavior similar to that observed with dispersions in urea solution.

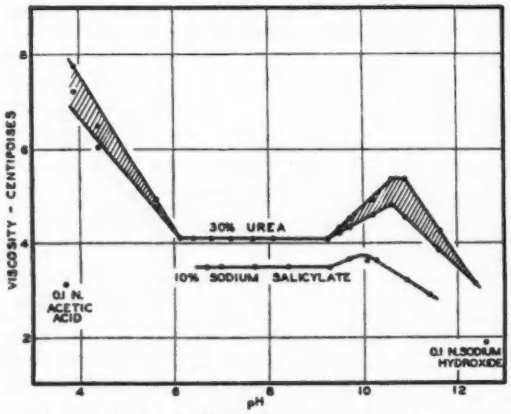

FIG. 3. *Effect of hydrogen ion concentration on the viscosity and stability of dispersions.*

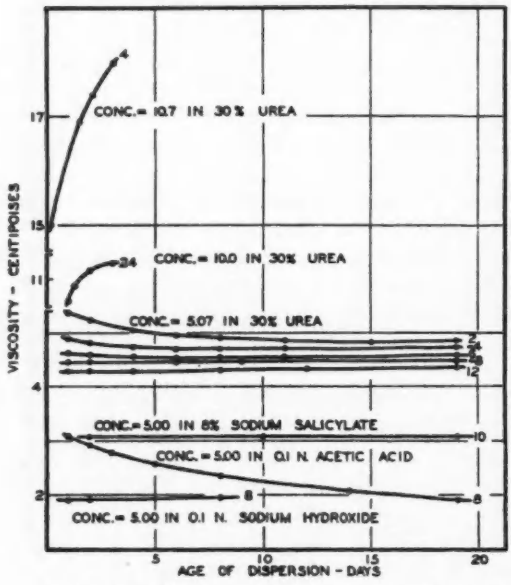

FIG. 4. *Effect of storage at 0° C. on the viscosity of dispersions.*

The viscosity and pH of one-day-old dispersions in sodium hydroxide and acetic acid are also plotted in Fig. 3. The viscosity of gluten in these reagents is not comparable with that obtained in the neutral solvents, but it is interesting to note that the pH of a dispersion in sodium hydroxide is beyond the unstable range on the alkaline side, while the pH of an acetic acid dispersion is probably about the middle of the unstable range on the acid side. This may account for the marked difference in the stability of dispersions in alkali and acid (Figs. 4 and 5), a point which will be discussed in the next section.

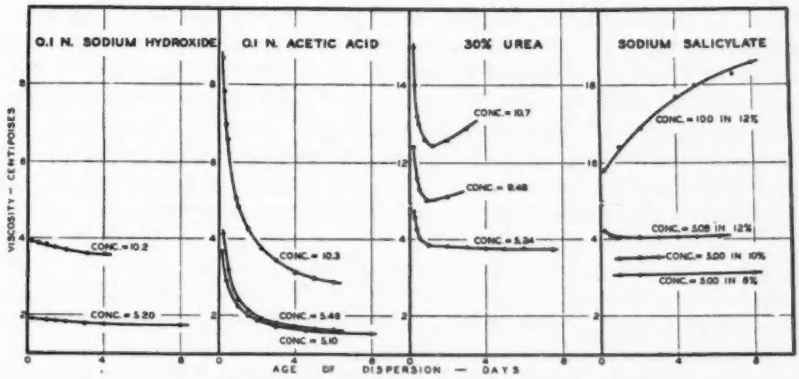

FIG. 5. *Effect of storage at 25° C. on the viscosity of dispersions.*

Changes Following Dispersion

In order to gain some idea of the changes which occur following dispersion, and also to determine the stability of the dispersed material, the viscosity changes occurring during storage at 0 and 25° C. were studied. Since the gluten had to be dispersed sufficiently to permit centrifuging before the viscosity could be measured, it had usually been exposed to the solvent for two hours or more before the first observation was made. The study of the viscosity changes occurring at 0° C. was further complicated by the fact that gluten would not disperse in urea solutions at this temperature. In order to have the results in all solvents comparable it was necessary to effect complete dispersion at 25° C. before placing the dispersions in ice water. Periodic measurements of the pH of the dispersions were made during storage. The maximum change observed was a decrease from pH 12.5 to pH 12.0, which occurred in dispersions in sodium hydroxide during eight days of storage at 25° C. Dispersions in acetic acid and sodium salicylate showed no significant change, while the pH change of dispersions in urea was less than 0.20. It is evident from Fig. 3 that this change could not have had any significant effect on the viscosity.

In the experiment on the effect of storage at 0° C., dispersions in urea solution were studied more extensively than those in other solvents. Both concentrated (10.0 mgm., or more, of protein nitrogen per gm.) and dilute (5.00 mgm. of protein nitrogen per gm.) dispersions were employed, the latter being centrifuged when 1.2 hr. old and portions placed on ice after 2, 4, 6, 8, and 12 hr. of storage at 25° C. in order to study the effect of these treatments. In this experiment the first viscosity measurement was made when the dispersion was one day old.

The viscosity changes with time of storage at 0° C. in all four solvents are shown in Fig. 4, all times being taken from the moment the wet gluten was placed in the dispersing reagent. The protein concentrations, in terms of mgm. of protein nitrogen per gm. of dispersion are given in the figure,

while the number at the end of each curve gives the number of hours that the dispersion was held at 25° C. prior to storage at 0° C. It was found impossible to follow the viscosity changes of a dispersion in alkali for more than eight days, as a slight precipitate invariably formed about this time. It is evident from the figure that the viscosity of the dilute dispersions in the neutral solvents is higher than that of dispersions in acid and alkali. In urea the viscosity decreases with increasing length of the initial storage period at 25° C. The viscosity of dispersions in sodium hydroxide, sodium salicylate and urea, when held initially for a period of six hours or longer at 25° C., remains practically constant, while that of dispersions in acetic acid decreases throughout the entire period. In contrast to these results obtained with dilute dispersions, the concentrated dispersions in urea solutions show a marked increase in viscosity during a four-day period at 0° C.

The viscosity changes which occur during storage at 25° C. are shown in Fig. 5. In all cases the time was measured from the moment the gluten was placed in the solvent. Again the viscosity of dilute dispersions in alkali and acid is much lower than of those in the neutral solvents. The viscosity of dispersions in acetic acid shows a marked decrease during eight days of storage at 25° C., the greatest decrease occurring in the first two days. A dilute dispersion in urea also shows a marked viscosity decrease during the first day, after which it remains constant. Dispersions of similar concentration in sodium hydroxide and 12% sodium salicylate show a very slight decrease in viscosity at first, and then remain constant for the rest of the period. Concentrated dispersions in sodium hydroxide and acetic acid decrease in viscosity during the entire period, but the fall-back is much greater in the latter solvent. In urea solutions the concentrated dispersions decrease in viscosity during the first day, but instead of remaining at a constant level, as with dilute dispersions, the viscosity later increases. Concentrated dispersions in sodium salicylate increased in viscosity throughout the period studied.

TABLE VI

PROTEIN NITROGEN SALTED OUT BY MAGNESIUM SULPHATE FROM DILUTE DISPERSIONS STORED AT 25° C.

Solvent	$MgSO_4$ added to 10.0 ml.	Age of dispersion when salt added, hr.	Protein nitrogen precipitated,	
			mgm.	%
30% urea	2.75 ml. of 20%	4	12.2, 14.6	24.4
		46	6.1, 7.4	12.3
8% sodium salicylate	1.50 ml. of 5%	10	15.3, 15.1	27.7
		48	12.5, 12.2	22.5

A number of salting-out tests were conducted on dilute dispersions in the neutral solvents during storage at 25° C. The results given in Table VI show that there was little difference between the amount of gluten salted

out from dispersions in sodium salicylate that were 10 and 48 hr. old. Reference to Fig. 5 suggests that the viscosity decrease over this period was also small. In urea solution the amount salted out after 46 hr. was considerably less than that obtained after 4 hr. and, as there was also a large viscosity decrease during this period (Fig. 5), it appears that the decrease in viscosity is due to a continuation of the dispersing action of the solvent rather than to a dehydration, for, had the latter reaction predominated, it might reasonably be expected that more, rather than less, of the gluten in the older dispersions would be salted out.

Recovery of Gluten from Various Solvents

An attempt was made to recover the gluten by salting out after prolonged dispersion, in the hope of obtaining more definite evidence as to which of the four solvents caused the least change in its character. The dispersions employed contained 5.00 mgm. of protein nitrogen per gm. and had been stored at 25° C. for three weeks. By this time a slight precipitate had already formed in the dispersion in sodium hydroxide. The dispersions were salted out by the addition of magnesium sulphate solution, those in acid and alkali having been previously neutralized.

The dispersion in sodium hydroxide became cloudier on adding the salt solution but no additional precipitate formed, nor could that which had previously appeared be matted into a gluten ball. Those in acetic acid and urea yielded a precipitate which was recovered by centrifuging and decanting the liquid. No matter how carefully the precipitate from the dispersion in acetic acid was handled it was impossible to mat it into a gluten. The precipitate from the dispersion in urea matted to form a gluten which was smooth, but inelastic and lacking in resilience. The dispersion in sodium salicylate yielded a gluten which came down without centrifuging and was smooth, but tough and inelastic. These observations indicate that the solvents fall in the following order with respect to decreasing effect on the protein: sodium hydroxide, acetic acid, urea, and sodium salicylate.

Viscosity of Glutens from Different Flours

A further comparison of the effect of the various solvents on gluten was made by studying the viscosity of dispersions of several glutens of widely different quality. The flours used in the first experiment included one of high baking strength milled from a sample of Marquis wheat, one of intermediate strength milled from Garnet, and a weak commercial pastry flour. All of the dispersions were centrifuged when six hours old, and analyzed. When from 9–11 hr. old, they were diluted to 5.00 mgm. of protein nitrogen per gm. A temperature of 25° C. was maintained during preparation and storage. The viscosity was determined when they were 1, 2, 3, and, in some cases, 4 days old.

The results given in Table VII show that the dispersions of the three glutens in urea solution had different viscosities, which remained practically constant over a three-day period, and which were correlated to some extent

TABLE VII

VISCOSITY OF DISPERSIONS OF GLUTEN OBTAINED FROM FLOURS OF DIFFERENT BAKING STRENGTH

Solvent	Flour	Viscosity, centipoises			
		1 day old	2 days old	3 days old	4 days old
0.1 *N* sodium hydroxide	Marquis A	1.73	1.69	1.68	
	Garnet	1.74	1.71	1.68	
	Commercial pastry	1.70	1.67	1.66	
0.1 *N* acetic acid	Marquis A	2.29	1.92	1.78	1.71
	Garnet	1.97	1.74	1.66	1.60
	Commercial pastry	2.16	1.85	1.73	1.66
30% buffered urea	Marquis A	4.04	4.06	4.04	
	Garnet	3.27	3.23	3.22	
	Commercial pastry	3.19	3.09	3.09	
10% sodium salicylate	Marquis A	3.47	3.51	3.59	
	Garnet	2.83	2.86	2.93	
	Commercial pastry	2.93	2.91	2.95	

with baking strength. The gluten from the strong Marquis flour exhibited a significantly higher viscosity in sodium salicylate than did glutens from the other flours. These had about the same viscosity when three days old, although the gluten from the Garnet flour had the lowest viscosity on the first day. This suggests that glutens retain their individuality to a lesser degree in sodium salicylate than in urea solution. The viscosity of all of the dispersions in sodium hydroxide was essentially the same. In acetic acid the three dispersions differed in viscosity when one day old, but on the fourth day, when the viscosity fall-back was practically complete, all had essentially the same viscosity. It is concluded therefore that dispersion in the neutral solvents caused less drastic changes in the original gluten than dispersions in acid and alkali.

Since the viscosity of gluten dispersed in urea solutions, and to a lesser extent that of gluten dispersed in sodium salicylate solutions, decreases with the baking strength, it appears that these solvents are not capable of dispersing a strong gluten to the same extent as a weak one. Although a study of gluten quality was not contemplated in this investigation, the foregoing results suggested that the viscosity of a dispersion in a neutral solvent might be used as a measure of quality. In order to study the point further the gluten from four flours, which differed less in baking strength than those previously used, was dispersed in the two neutral solvents and the viscosity of the dispersions determined as before. The results obtained, together with the protein content of the wheat from which the flour was milled and the loaf volume and texture score resulting from an experimental baking test, are given in Table VIII.

The actual baking quality as judged by the loaf volume, places the flours in the order in which they are listed. It cannot be said, however, that these

TABLE VIII

VISCOSITY OF GLUTEN DISPERSED IN THE NEUTRAL SOLVENTS IN RELATION TO THE BAKING STRENGTH OF FLOUR

Flour	Protein in wheat[1], %	Baking test by bromate formula[2]		Viscosity, centipoises					
		Loaf volume, ml.	Texture score[3]	In 30% urea			In 10% sodium salicylate		
				1 day	2 days	3 days	1 day	2 days	3 days
1	15.3	687	7.5	3.38	3.30	3.30	2.98	2.99	3.02
2	14.8	648	5	3.35	3.29	3.25	2.99	2.94	2.94
3	15.4	616	3	3.26	3.12	3.08	3.00	3.00	3.02
4	16.0	588	5.5	3.22	3.15	3.08	2.90	2.84	2.87

[1] *Expressed on a 13.5% water basis.*
[2] *100 gm. of flour on a 13.5% water basis.*
[3] *Perfect score = 10.*

differences in baking quality are due entirely to the quality of the gluten. The protein content of Flour 2, for instance, is lower, and that of Flour 4 higher, than those of Flours 1 and 3. Furthermore, such factors as diastatic activity, etc., which were not determined in the flours studied, also affect the loaf volume. The viscosity of the gluten from the last two flours was lower than that from the first two, when dispersed in urea solutions, while the viscosity of the last flour was lower than that of the others in sodium salicylate solution. Determination of the viscosity of gluten in a neutral solvent would thus seem to be of doubtful value for predicting the quality of the protein. The technique is rather time-consuming and laborious, and involves certain processes, such as gluten washing, which are difficult to standardize rigidly when the glutens vary in strength. Nevertheless, this type of viscosity determination appears preferable to those performed on acidulated flour-in-water suspensions. The complicating effects introduced by the presence of starch, electrolytes, and varying protein concentrations are removed on washing the gluten and adjusting the protein concentration after dispersion, and in addition, the viscosity is measured at a pH at which the protein is reasonably stable.

4. Discussion of Results

It has been shown that the viscosity of gluten dispersions in urea and sodium salicylate is much higher than that of dispersions of the same concentration in alkali or acid. This is attributable in part to the higher viscosity of the solvents themselves, but the application of Kunitz's equation to the relative viscosity of the dispersions shows that the equivalent hydrodynamic volume of the gluten is larger in the neutral solvents than in acid or alkali. This indicates either a greater solvation or a lower degree of dispersion in the former solvents. The latter seems more probable.

The changes in the viscosity with hydrogen ion concentration must represent some change in the state of the dispersed gluten. It seems unlikely

that either the electro-viscous effect, or differences in solvation alone could account for the nature and magnitude of these. The high unstable viscosity maxima may, however, reasonably be attributed to an extension and swelling of the particles preceding their further dispersion into the smaller units which constitute the stable system at extreme hydrogen ion concentrations.

These results are of interest in connection with viscosity tests of acidulated flour-in-water suspensions. Since Sharp and Gortner's (21) work many investigators have tried, with varying degrees of success, to use this test as an index of protein quality. Difficulty has been experienced in standardizing the procedure rigidly enough to give reproducible results, although Reiman (20) found that with proper precautions, particularly with respect to the lactic acid, results could be duplicated. The present work shows that the region of maximum viscosity is also the region of maximum instability and probably explains the difficulty of obtaining consistent results. Measurements at lower temperatures, however, where the proteins are more stable, may prove more satisfactory.

The viscosity changes which occur during storage at 0° C. and 25° C. give some idea of the time required for complete dispersion, and the stability of the resulting system. The relatively constant viscosity values obtained in sodium hydroxide suggest that the dispersing action of this solvent is extremely rapid, and that it is practically complete before an observation can be made. Acetic acid appears to disperse the gluten more slowly and a constant viscosity level was never reached. The behavior of concentrated dispersions in the neutral solvents indicates that the initial dispersion reaction is followed by some form of aggregation or flocculation. This second type of reaction was never evident in dilute dispersions in any of the solvents or in the concentrated dispersions in acid and alkali.

In urea solutions the viscosity level reached by dilute dispersions was considerably higher at 0° C. than at 25° C. It seems likely that this can be attributed largely to increased dispersion at 25° C. rather than to increased solvation at the lower temperature, especially since the fall-back at 25° C. appears to be irreversible, there being little tendency for the viscosity to increase on storage at 0° C. after storage at 25° C. Furthermore, the dispersion is more resistant to salting out after storage at 25° C. than it was originally, a condition scarcely compatible with a more dehydrated state of the gluten particles. The higher viscosity observed at 0° C. consequently indicates that the degree of dispersion in urea solutions is dependent on the temperature.

Although these experiments give considerable information regarding the changes which occur in the different solvents during dispersion, they provide no conclusive evidence as to which is the best solvent for gluten. If McBain's (18) criterion, that the best solvent is the one giving the least viscous dispersion, is accepted, then sodium hydroxide and acetic acid are superior to the neutral solvents. This criterion, based mainly on colloidal considerations, however, is scarcely valid for protein dispersions where, quite apart from the

degree of dispersion, irreversible physical and chemical changes may be brought about by the solvent. On the other hand, if the properties of gluten are determined to some extent by its state of aggregation, the dispersions in solvents causing a low degree of dispersion, as shown by a high viscosity, may be expected to represent the properties of the original substance more closely than those in solvents causing a high degree of dispersion. If this reasoning is correct, then the neutral solvents are better than acid or alkali. As an increase in viscosity occurred in concentrated dispersions in the neutral solvents, it may be contended that this evidence of coagulation marks them as inferior. This, however, is not a valid criticism since an alteration in the proteins, such as denaturation, may occur in alkali and acid, although coagulation may be prevented.

It is concluded from the experiments on gluten recovery, and on the viscosity of gluten from flours differing in baking strength, that dispersion in neutral solvents alters the gluten less than dispersion in acid or alkali. The main difference between the two classes of solvents appears to be that alkali and acid effect a higher degree of dispersion. Whether or not this is accompanied by a chemical change is unknown, but gluten after dispersion in alkali or acid appears to have lost its original properties, whether judged by the salted-out product or by the viscosity behavior of a series of glutens known to have differed originally.

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ROOT ROT OF GINSENG IN ONTARIO CAUSED BY MEMBERS OF THE GENUS *RAMULARIA*[1]

By A. A. Hildebrand[2]

Abstract

Two destructive diseases of the root of ginseng, *Panax quinquefolium* L., have assumed economic importance in the ginseng-growing districts of Ontario. The more destructive of the two diseases, commonly known as the disappearing-rot, is characterized by the fact that affected roots may, in a relatively short time, either completely disappear in the soil or leave as evidence of their presence only a peridermal shell enclosing fragments of vascular tissue. The disease, which is non-systemic, is caused by at least three representatives of the genus *Ramularia* of the Fungi Imperfecti, one of which has been identified as *Ramularia panacicola* Zins., and the other two being new to science, are described as new species for which are proposed the binomials, *Ramularia mors-panacis* and *Ramularia robusta*. No sexual stage has been observed in connection with the three species. They appear to persist in the soil indefinitely either saprophytically or in a dormant condition.

The name rust has been applied to the other serious but less destructive disease because of the occurrence on the surface of affected roots of superficial, rust-colored lesions. The cause of the rust disease has not been definitely established but the evidence thus far suggests that it is probably also caused by representatives of the genus *Ramularia*.

Rotation and rigid sanitation are the only control measures suggested, resistant varieties not having been encountered.

In the present work a large number of additional representatives of the genus, different from each other and from the isolants from ginseng, have been obtained from tissue isolations from similar lesions on various other hosts. Cross-inoculations indicate variations in pathogenicity among the isolants and specificity in host relations. This demonstration of the universal occurrence and parasitic capabilities of soil-inhabiting representatives of the genus *Ramularia* changes the concept of the genus and adds a most important member to the group of facultative parasites associated with root troubles of plants.

Introduction

During recent years growers of American ginseng, *Panax quinquefolium* L., in various parts of Ontario, have found it increasingly difficult to bring to maturity a plant whose root, which is the article of commerce, requires at least five years' growth before it reaches a desirable marketable stage. Of various diseases which are primarily responsible both for impairment of quality and for reduction in yield, two which attack the roots are pre-eminent. The more serious of the two diseases is variously known as the brown rot, the disappearing-rot or, more simply, the rot, while to the other disease has been ascribed the name "rust". The increasing prevalence of one or of both of these diseases in the ginseng-growing districts of Ontario has created a problem of considerable economic importance. The present paper reports the results of investigations carried out in an attempt to discover (i) the cause of the diseases, (ii) the phenomena associated with their occurrence and spread, and (iii) successful methods of control.

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Review of Literature

Diseases of ginseng have been the subject of scientific research since 1904, when Van Hook (10) published the results of investigations carried out in New York State, where within little more than a decade after the plant had been brought under cultivation, diseases had already become serious in almost every ginseng-growing section. Among other diseases Van Hook mentions an end rot of seedlings characterized by a rotting away of the taproot, beginning usually at the lower end. The cause of this disease was not determined and only a very meagre description is appended. Whetzel and Rankin (17) in 1909, and Whetzel and Osner (18) in 1910, having investigated a disease of older roots, which had become widespread and destructive not only in New York but in other ginseng-growing states, and to which had been ascribed the name "rust" or "fibre-rot", concluded that the disease was "but another form of the end rot of seedlings". Whetzel and Rosenbaum (19) in 1912, reaffirmed the above conclusion with regard to this disease, stating after giving a detailed description of symptoms as they appeared on seedlings and on older roots, that "while the absolute proof of the common cause of these different symptoms on seedlings and older roots has not yet been established, the evidence thus far accumulated indicates that they are one and the same thing and may be, at least for the present, referred to as the rust". They also considered the evidence at this time as pointing to the fungus *Thielavia basicola* (B. and Br.) Zopf., as the causal organism of the disease. By 1916 the brown rot or rust was prevalent throughout the ginseng-growing districts of Wisconsin, and Brann (2) records attempts at control by steam sterilization of soil. According to Whetzel, Rosenbaum, Brann and McClintock (20) 1916, all efforts to find a definite organism associated with the disease had failed, and the opinion was expressed that "doubtless several of the root rots (especially those caused by *Alternaria panax* and *Thielavia basicola*) are commonly included with other brown rots and rusty discolorations of the roots under the name rust". Previous to the work of Zinssmeister (25) in 1918, therefore, the term rust had been applied more or less indiscriminately to diseases which, though they may have resembled one another in their general symptomatology, were undoubtedly caused by different organisms. Zinssmeister for the first time employed the term rust to specify a definite root disease. Isolations made from material received from two widely separated states, New York and Wisconsin, yielded in the majority of cases, isolants of *Ramularia*. The latter were resolved into two species which, being new to science, were described and named, one being designated *Ramularia destructans*, the other *Ramularia panacicola*. Both species were found to be pathogenic on dormant roots and evidence was adduced to show that the disease could develop during the dormant season.

Berkeley (1) in 1927, found evidence of the presence of two troubles in certain of the ginseng-growing districts in Ontario; one, of the nature of a soft rot, the other, of a scabby or rusted condition of the roots. Isolations from diseased material yielded several organisms including *Ramularia* spp.

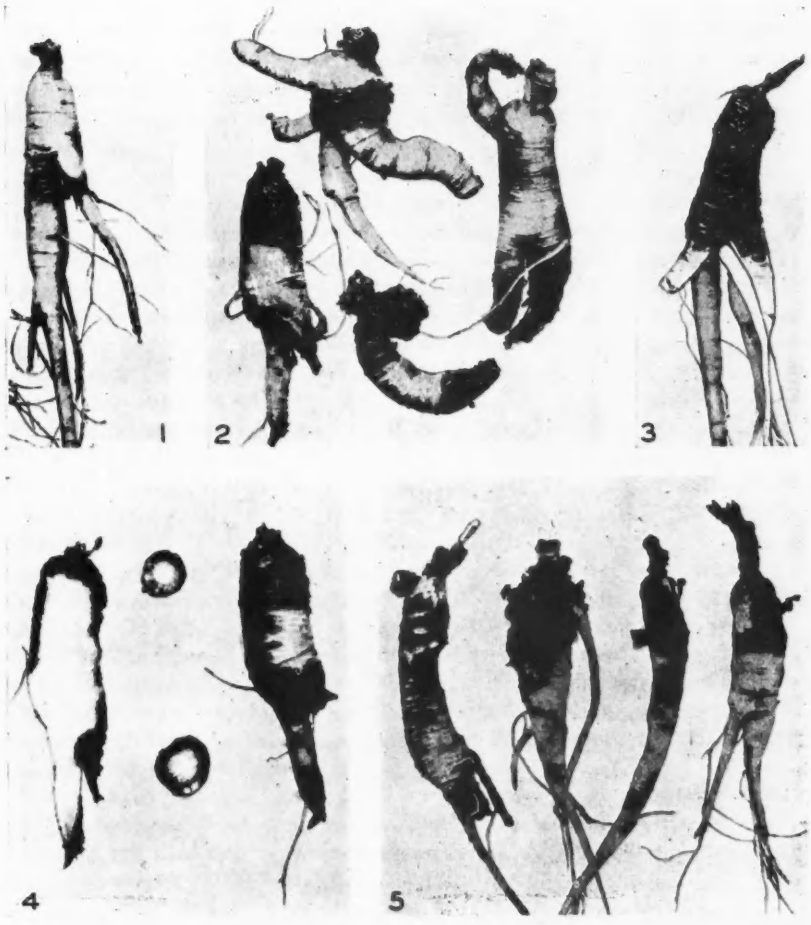
Nakata and Takimota (5) in 1923, published the results of studies of diseases of Asiatic ginseng *Panax ginseng*, in Korea, where the production of this crop assumes an important place in Korean agriculture. Two diseases of the root, red rot and amber-colored rot, both of bacterial origin, are of outstanding importance, being prevalent in all regions of Korea where ginseng is cultivated. Whetzel (21) as recently as 1928 seemed to hold the opinion that the rust in America as defined by Zinssmeister and shown by him to be of fungous origin, is the same disease as the Korean red rot, which, according to the Japanese investigators mentioned above, is a bacterial disease.

Symptoms of the Diseases

Rot

The rot affects directly the parts of the plant that are underground, namely, (i) the root, (ii) the perennial stem, and (iii) that portion of the current-year stem extending upwards from the point of attachment on the perennial stem to the ground level. The most striking indications that the disease is active in a given stand are, first, the failure of the plants to come up in the spring, and second, the wilting of the aerial parts during the growing season. The appearance of a root affected with rot depends on the stage of development of the disease. In the early stages of infection there appear on the root at any point on its surface, either on the main taproot or on the laterals, small, brownish, discolored areas (Plate 1, Fig. 1). At this stage there is no rupturing or distortion of the periderm other than a slight wrinkling, and if the root is cut across, the lesion is found to be quite shallow. The lesions increase rapidly both in surface extent and in depth of penetration (Plate 1, Figs. 2, 3 and 4). If the periderm is removed, the underlying, reddish-brown, diseased tissue is found to be spongy and moist but not water-soaked. Even in advanced stages, the tissues of the root are not resolved into the water-soaked, amorphous masses typical of soft rots. In later stages the perennial stem and the crown of the root may be completely rotted away. This is almost invariably indicated by the ease with which the now wilted stem can be detached. In other cases the root fibres and smaller laterals may have completely disappeared, or, very often, the lower part of the main root itself, or that of one of its larger laterals, may be rotted off, the combined effect being to leave the root in a bare and stubby condition (Plate 1, Fig. 2). In the final stages, very often all that remains of the root are remnants of the peridermal shell enclosing a few fragments of vascular tissue. Finally not a trace of the root is to be found. There does not seem to be any correlation between age and susceptibility to attack, the disease having been observed in seedlings and in roots of all ages up to six years.

Wilting of aboveground parts is sooner or later an indication of a rotted condition of the roots. The extent to which a root may be rotted before the sudden collapse of the aerial parts which have given no evidence of the presence of the disease, is remarkable. In the warmer and drier part of the growing season, plants with rotted roots may wilt during the hottest part of the day and recover towards evening. Such recoveries, however, are only



FIGS. 1, 2 and 3. *Roots naturally infected with disappearing-rot showing typical lesions in early and later stages of the disease.* FIG. 4. *Sections of rotted roots showing depth of lesions.* FIG. 5. *Naturally infected rusted roots. Note areas where periderm has sloughed off exposing healthy tissue.*

temporary and soon the wilting becomes permanent. Sometimes, in older plants, one of the compound leaves, three of which arise in a whorl from the apex of the slender, upright stem, will show signs of wilting while the other two appear perfectly healthy. Examination of the root in such cases very often shows that a main branch has rotted away, thus cutting off the water supply from one side of the plant with consequent wilting of the parts more directly affected. Changes in color from the normal dark green of the healthy foliage sometimes accompany wilting but, since similar color changes often result from causes other than the rot, they are of uncertain diagnostic value.

Rust

The appearance of rusted roots also depends on the stage of development of the disease. In earlier stages discolored areas of varying size are to be observed on the surface of the root. These are due to the presence of more or less densely aggregated, slightly raised, reddish-brown spots, which, in turn, vary in size from minute dots whose individuality is only discernible with the aid of a hand lens, to spots of macroscopic size. The latter are irregular in shape and are often extended around the circumference of the root, seeming to follow the depressions of the circular wrinkles which characterize the surface of ginseng roots. At this stage, whatever the size of the individual spots comprising a diseased area, they are quite superficial and, being slightly raised, give to the surface of the affected area a slightly roughened appearance. In later stages, the smaller spots grow larger and coalesce to form definite and continuous lesions, which, though they may involve considerable surface area of the root, penetrate to the depth of a few cell layers only. Owing to the rupturing and sloughing-off of the periderm over considerable areas, the roots at this stage present a decidedly scurfy appearance (Plate 1, Fig. 5). When scraped slightly with a knife blade or even rubbed with the thumb, the diseased tissue is easily detached, leaving exposed the white, healthy tissue beneath. While rust lesions, as in the case of those of the rot, may be found on any part of the root, they appear to be of more common occurrence in the region of the crown, and often extend upwards to involve the perennial stem.

Plants whose roots are badly rusted are often slightly stunted, and mature earlier than those whose roots are healthy. These are almost the only indications of the presence of the disease so far as the aboveground parts are concerned.

Economic Importance and Distribution of the Disease

In the vicinity of Waterford, Norfolk County, where ginseng growing is carried on more extensively than elsewhere in Ontario, the disappearing-rot has caused greater loss than any other disease. From small centres of infection which appear variously and unexplainably scattered throughout the gardens, the disease spreads radially outward, occasioning complete destruction of roots as it advances. Probably one of the chief factors in the rapid spread of the disease, especially in the case of older roots, is the close proximity of the latter to one another, their fibres intertwining in the soil

to form a more or less continuous host-bridge for the spread of the pathogen. Once the soil has become infested, it cannot be sown to ginseng again for an indefinite period and many gardens have had to be abandoned on account of ginseng-sick soil. Although less destructive than the rot, in that it does not occasion the complete disappearance of the root, nevertheless, by rendering large quantities of roots marketable only as culls, the rust also causes heavy losses in certain of the gardens of Norfolk County.

In the parts of Peel and Dufferin Counties which rank next in importance as ginseng-producing centres, a survey has shown that the rust, but not the rot, is prevalent in certain gardens where it is occasioning considerable losses.

Both diseases have been found in widely different types of soil ranging in texture from heavy clay loam, through various intergrades of loam and humus content, to light, sandy loam. Once established, both diseases are serious, seemingly regardless of type of soil or meteorological conditions.

Isolations from Diseased Roots

Rotted Roots

Isolations from diseased roots were made over a period of four years both from material collected personally and from specimens forwarded to the laboratory from various parts of Ontario. In the case of rotted roots showing early stages of infection, a portion of the root showing a small lesion was excised and washed under running water for from 5 to 15 min., a small, fairly stiff-bristled brush being used to facilitate the removal of extraneous material. After final rinsing in sterile water, small bits of tissue were removed from the periphery of the lesion, and transferred to tubed slants. Roots showing more advanced stages of the rot were first washed under running water and then surface-sterilized by immersion in mercuric chloride (1/1000) for periods varying from one to four minutes, depending on the depth of the lesion. After rinsing finally in sterile water the root was cut across through the lesion and from the innermost depth of the latter, where diseased and healthy tissue merged into one another, bits of tissue were removed and transferred to tubed slants. In the case of the more severely diseased roots, macerations of diseased tissue were made in sterile water, after washing and surface sterilization. The suspension was then streaked on plates of solid medium. Potato-dextrose agar acidified to the extent of 2 drops of 25% lactic acid per 15 cc. of medium was employed throughout.

Many different organisms were obtained from rotted roots. Of most frequent occurrence in the aggregate were isolants of *Ramularia*, some of which appeared early in the investigations, others comparatively recently, but all of which were resolved into five different strains or growth forms, for convenience tentatively designated as strains *P*, *M*, *R*, *S* and *B*. The roots from which strains *P* and *M* were isolated were obtained from the same garden, while strain *R* was isolated from roots obtained in another garden about a mile distant. Strains *S* and *B* were isolated from diseased specimens forwarded to the laboratory from two widely separated districts in Ontario.

Second in frequency of occurrence, and in some series of isolations predominating numerically, were different strains of *Fusarium*. Certain of these,

as well as the five strains of *Ramularia*, were obtained in pure culture for subsequent tests as to their pathogenicity. More or less sporadically all through the investigations there also appeared in culture representatives of the genera of fungi so frequently reported in studies of this kind, namely, *Trichothecium*, *Penicillium*, *Aspergillus*, *Verticillium*, *Alternaria* and *Mucor* spp.

Rusted Roots

Rusted roots were much more difficult to work with than rotted roots. The porous and superficial nature of the lesions and the ease with which the infected tissues became detached, rendered it almost impossible either to surface-sterilize the roots or to wash them sufficiently to remove more than the coarser soil particles. The roots were washed as thoroughly as possible through successive changes of sterile water, and transfers of diseased tissue were made to acidified potato-dextrose agar. By this method certain fungi were found consistently enough in association with rust lesions to suggest a possible causal relation. These included five different strains of *Ramularia* (none of which was identical with any of those isolated from rotted roots), several different strains of *Fusarium*, and one species each of *Sporotrichum* and *Hormodendrum*. Pure cultures of all these organisms were obtained by the monospore method. As was the case in plantings from rotted roots, numerous other presumably saprophytic soil-inhabiting fungi appeared in isolations from rusted roots.

Infection Experiments

Several attempts were made to grow ginseng in experimental plots at the laboratory at St. Catharines but with indifferent success, consequently the infection experiments for the most part were carried out in the gardens at Waterford, Norfolk County, where plants of all ages were made available through the co-operation of several growers of the district.

Series A. Involving Organisms Isolated from Rotted Roots

Experiment 1, 1929-30, Garden A, Waterford

Using pure cultures of *Ramularia M*, and two strains of *Fusarium*, *F* and *D*, the three organisms which up to that time had been most consistently isolated from rotted roots, the first infection experiment was carried out, Sept. 5, 1929, in a bed of apparently healthy four-year-old roots growing in heavy, clay-loam soil. Ninety carefully selected roots were involved, 15 being inoculated with *Ramularia M*, 15 with *Fusarium D*, 15 with *Fusarium F*, 15 with a mixture of cultures of *Ramularia M* and *Fusarium D*, 15 with a mixture of cultures of *Ramularia M* and *Fusarium F*, while 15 served as checks. In 8 of the roots in each group of 15, the inoculum was inserted into artificial injuries. The remaining 7 roots, without injury, were sprayed with spore suspensions of the different organisms.

The results of the experiment became known May 21, 1930. The 45 roots which had been inoculated with *Ramularia M* either alone or in combination with each of the two strains of *Fusarium*, had disintegrated to the extent

that only fragments of peridermal shell could be found. Of the 30 roots inoculated with the two strains of *Fusarium* alone, 7 could not be found and 6 others showed various stages of rot. Three of the latter were roots which had been injured when inoculated and it was noted that the rot had not started at the point of injury. The remaining 17 roots had remained healthy except that several of those which had been injured showed at the point of injury small but definite diseased areas, none of which, however, were typical disappearing-rot lesions. Nine of the 15 checks were found to have remained healthy but the remaining 6 had badly rotted roots. The latter were all adjacent to the part of the bed which had not been used in the experiment and subsequent investigation showed that many of the plants in this part of the bed had rotted roots. Specimens of these were taken to the laboratory and from them was isolated not only *Ramularia M* but, in addition, a new and closely related representative of the same genus which was designated *Ramularia P*.

The almost complete disappearance of all roots which had been inoculated with *Ramularia M* either alone or in combination with the two strains of *Fusarium*, as contrasted with the survival of the majority of those which had been inoculated with the *Fusarium* strains alone, strongly suggested *Ramularia M* as a possible causal agent of the disease. Indications were also given that the pathogen was not a wound parasite and that the disease was capable of rapid development at relatively low soil temperatures. At the time the experiment was begun, in Sept., 1929, not one of the 90 roots involved showed any signs of disease. In many cases, however, since only a small area of the root was exposed, it is quite possible that, on many of them, incipient infections were already present on parts not uncovered. In view of our later knowledge of the rapidity with which the disease can develop and spread, it seems equally possible that infections could have taken place after the commencement of the experiment. In any case, the appearance of disease in the check plants, together with the impossibility of completing the rules of proof in the case of the suspected organism, made it necessary to repeat the experiment.

Experiment 2, 1930, Gardens A and B, Waterford

A second infection experiment was carried out in the field June 17, 1930, in two gardens about a mile apart where the soils were of remarkably different texture and consistency, that in garden A—where the first experiment had been carried out—being a heavy clay loam, that in garden B consisting of an easily workable, light loam with high humus content and closely resembling forest soil in appearance and texture. The plants in garden A were five years old, those in garden B, three. The organisms used as inoculum were *Ramularia M*, the more recently isolated *Ramularia P* and two strains of *Fusarium F*, which had been used in the first experiment, and a new strain, *H*, which had meanwhile appeared consistently in isolations from rotted roots. In this experiment inoculation by spore suspension was omitted, all the roots being injured and the inoculum inserted into the injury. The data in connection

with this experiment and the results as observed Aug. 18, 1930, 52 days after inoculation, are summarized in Table I.

TABLE I

SUMMARY OF RESULTS OF INFECTION EXPERIMENT 2, JUNE 17–AUG. 18, 1930

Garden	Age of roots, years	Soil type	Organism		No. roots injured and inoculated	Injured checks	Results
			Fusarium	*Ramularia*			
A	5	Heavy clay loam	- - - -	Strain *P*	16	- - - -	100% disappearing-rot infection
A	5	Heavy clay loam	Strain *H*	- - - -	16	- - - -	100% typical *Fusarium* infection
A	5	Heavy clay loam	- - - -	- - - -	- - - -	12	73% healthy
B	3	Light loam	- - - -	Strain *M*	16	- - - -	100% disappearing-rot infection
B	3	Light loam	Strain *F*	- - - -	16	- - - -	100% typical *Fusarium* infection
B	3	Light loam	- - - -	- - - -	- - - -	12	83% healthy

On Aug. 18, all 16 roots in garden A, which had been inoculated with *Ramularia P* were found to be typically rotted, the majority of them so severely that it was difficult to remove them intact from the soil. Practically identical results were obtained with *Ramularia M* in garden B. Re-isolations from roots from both gardens were disappointing in that cultures of *Fusarium* predominated over those of *Ramularia P* and *Ramularia M* in the ratio of five to three.

The results obtained in the case of roots inoculated with *Fusarium H* (garden A), and *Fusarium F* (garden B), closely approximated one another. In every case small but definite lesions developed around the point of injury, but these lesions differed so markedly from those typical of the disappearing-rot as to be clearly distinguishable from them.

Of the 24 check plants, three in garden A and two in garden B developed typical rot before the termination of the experiment. The rest remained healthy.

From the results obtained in this experiment it appeared that both *Ramularia P* and *Ramularia M*, when inoculated into artificially injured but otherwise healthy ginseng roots, were capable of producing symptoms as characteristic of the disappearing-rot as those resulting from natural infection. Apparently also the disease can develop rapidly in soils differing markedly in texture and content, under the relatively drier and warmer conditions which obtain in midsummer. The two strains of *Fusarium, F* and *H*, though slightly pathogenic, produced symptoms entirely different from those of the disappearing-rot. The predominance of strains of *Fusarium* over both *Ramularia P* and *Ramularia M* in isolations from lesions induced primarily by the latter organisms is most reasonably explainable on the basis of secondary invasion by a ubiquitous, saprophytic, soil fungus.

Experiment 3, 1930, Garden B, Waterford

The results obtained in the first two infection experiments had proved almost conclusively that the two forms of *Ramularia*, *P* and *M*, bore a causal relation to the disease, yet, in neither case, had the requirements of the rules of proof been fully satisfied. In the first experiment, re-isolations could not be made from inoculated roots because of the disappearance of the latter before the termination of the experiment. In the second experiment, although the two forms of *Ramularia* were recovered from inoculated roots, *Fusarium* spp. appeared in the majority of the re-isolations. A third experiment was undertaken with slight modifications in the technique which, it was thought, might render more certain the recovery of the primary causal organisms from inoculated roots. In the first two experiments, in cases where roots had been injured and inoculated, a small, moist pad of absorbent cotton was applied to cover the wound after the inoculum had been inserted. In the third experiment this procedure was repeated but, in addition, the smaller pad was covered with a second and larger one of dry non-absorbent cotton, which was pushed well down alongside the root. In this experiment uninjured roots also were inoculated with spore suspensions of the organisms. Sufficient soil was removed to expose a part of the root surface, a heavy spore suspension was applied by means of an atomizer and the soil was replaced. The organisms used were the two forms of *Ramularia*, *P* and *M*, and a new strain of *Fusarium*, designated *Fusarium* 148, which had been obtained in a high percentage of cases from roots inoculated with the two forms of *Ramularia* in Experiment 2. The complete data in connection with this experiment, beginning Aug. 18 and terminating Sept. 19, 32 days later, are summarized in Table II.

TABLE II

SUMMARY OF RESULTS OF INFECTION EXPERIMENT 3, AUG. 18–SEPT. 19, 1930

Organism	No. plants inoculated		Results of inoculation	Results of re-isolations
	Spore susp.	Injured		
Ramularia P	- - - -	8	100% disappearing-rot infection	*Ramularia P*, 61%; *Fusarium* spp. 39%
Ramularia P	8	- - - -	87% disappearing-rot infection	*Ramularia P*, 100%
Ramularia M	- - - -	8	100% disappearing-rot infection	*Ramularia M*, 85%; *Fusarium* spp. 15%
Ramularia M	6	- - - -	100% disappearing-rot infection	*Ramularia M*, 78%; *Fusarium* spp. 22%
Fusarium 148	- - - -	8	50% typical *Fusarium* infection	*Fusarium* 148, 100%
Fusarium 148	8	- - - -	100% healthy	
Checks	- - - -	6	100% healthy	
Checks	6	- - - -	100% healthy	

All 16 roots injured and inoculated with the two forms of *Ramularia*, showed typical disappearing-rot lesions in the region of injury 32 days after inoculation (Text-fig. 1). In the case of the roots inoculated with *Ramularia P*, the organism was recovered from 60.9% of the plantings as compared with *Fusarium* spp. in 39.1%. From the roots inoculated with *Ramularia M*, the organism was recovered in 85% of the plantings, while *Fusarium* spp. appeared

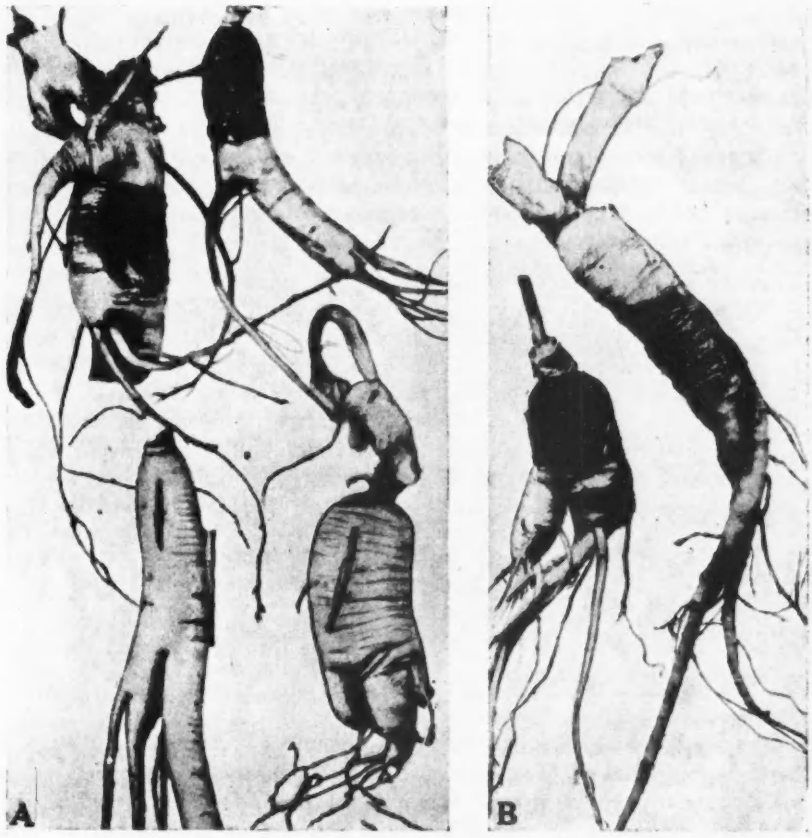


FIG. 1. *Ginseng roots artificially injured and inoculated with two species of Ramularia, 32 days after inoculation. A, Roots inoculated with Ramularia mors-panacis sp. nov., with corresponding checks. B, Roots inoculated with Ramularia panacicola Zins.*

in the remaining 15%. Of the 14 roots inoculated with spore suspensions of the two isolants of *Ramularia*, 13 showed typical rot lesions of varying size, none of which, however, penetrated as deeply into the tissues of the root as did those resulting from wound inoculations, but from all of which, as reference to Table II will show, the organisms were recovered.

Of the 16 roots inoculated with *Fusarium* 148, only four of the eight roots which had been injured developed lesions. These were confined to the immediate region of the injury and differed markedly from typical rot lesions. The roots to which the spore suspension of this organism had been applied remained healthy, as did all the checks.

The results of this third experiment leave little doubt as to the aggressiveness of the two forms of *Ramularia*, *P* and *M*, whether or not an infection court is provided by artificial means. Both strains are extremely virulent in their attack. Although undoubtedly the primary parasites, they are probably not alone responsible for the rapid disintegration and ultimate disappearance of the root. Metabiosis or succession of organisms undoubtedly plays an important part throughout the later phases of the disease.

Fusarium 148 is apparently only a weak wound-parasite on ginseng and together with strains *H*, *F* and *D*, can be eliminated as a primary causal agent of the disappearing-rot. They may all, however, be important later links in the metabiotic chain.

Experiment 4, 1930, St. Catharines

While Experiment 3 was in progress, a new isolant of *Ramularia*, morphologically quite different from either *M* or *P* appeared in plantings from rotted roots. The pathogenicity of this new isolant, designated *Ramularia R*, was tested on healthy, three-year-old plants growing in experimental plots at St. Catharines. The roots of 10 of these plants were injured and inoculated while 5 similarly injured roots served as checks. The experiment was of 25 days' duration. In the case of the inoculated roots, 100% infection was obtained, while all the checks remained healthy. *Ramularia R* was recovered in 90% of the plantings from the inoculated roots, *Fusarium* spp. in 7%, while representatives of *Penicillium*, *Alternaria*, *Rhizoctonia*, and *Rhizopus* spp., developed from the remaining 3% of the plantings. It was observed in this experiment and in others carried out subsequently that *Ramularia R*, though producing typical rot lesions, does not display the same degree of virulence as forms *M* and *P*.

Experiment 5, 1930-31, Laboratory and Greenhouse

The results obtained in the field experiments were checked by a series of laboratory experiments involving seedlings and older roots. Following surface sterilization by immersion for 30 min. in a solution of formalin, 1½ oz. of commercial formalin to 1 gallon of water, seeds were planted in artificially infested and non-infested soils, steam sterilized in both cases. Healthy roots, surface-sterilized by immersion for six to eight minutes in mercuric chloride (1/1000), followed by rinsing through several changes of sterile water were:

(i) Planted in artificially infested soil, steam sterilized prior to infestation.

(ii) Dipped in or sprayed with a spore suspension of the organism and planted in sterilized soil.

(iii) Injured, inoculated and planted in sterile soil.

The experiments fell naturally into two groups: (a) those in which there was no attempt at careful control of soil moisture and temperature; (b) those which were carried out under conditions of rigid control of these two variables.

Group (a). In this group of experiments the pathogenicity of the three strains of *Ramularia*, *M*, *P*, and *R*, and of two strains of *Fusarium*, *H* and *F*, was tested on roots but not on seedlings. Regardless of how the inoculum was brought into contact with the roots, the three isolants of *Ramularia* never failed to produce infection followed by typical rot symptoms. The two strains of *Fusarium* proved to be weakly parasitic on injured roots, the lesions produced by both strains closely resembling one another but differing from typical disappearing-rot lesions. In general, the results of these experiments closely approximated those obtained in the field.

Group (b). For experiments requiring more rigid control of environmental conditions, three units of modified Wisconsin constant-temperature tanks (8-container capacity each) were used. With this equipment it was possible to investigate, to some extent at least, the relation of soil moisture and soil temperature—probably the most important of several variables—to infection of roots and seedlings.

The soil, a light loam, was autoclaved for 3-hr. periods on each of three successive days. Soil sufficient to fill half the containers was adjusted to 50% of its moisture-holding capacity (hereinafter abbreviated to M.H.C.), while a similar quantity was adjusted to 70% of its M.H.C. Throughout the duration of the experiment—Feb. 19 to April 19, 1931—the containers were weighed daily and sufficient water was added to maintain the required moisture content.

Time and material did not permit the testing of more than one organism. *Ramularia P* was chosen arbitrarily. Preliminary studies had shown that this organism grew on potato-dextrose agar (dextrose, 2½%) at temperatures ranging from 2° to 26–28° C. The top six inches of the soil in half the containers was infested by mixing intimately with it, 60 gm. of oats on which the fungus had been grown for fourteen days.

The roots used in the experiment had been obtained from a garden, which, after a careful survey, appeared to be as free from disease as any in the Waterford district. The required number of roots, following surface sterilization, were planted both in infested and in non-infested soils, at each of the two different moisture contents.

The seeds used in the experiment were all carefully selected, only those being used whose germination was almost assured by the already partial rupturing of the endocarp. They were surface-sterilized and planted, as in the case of roots, in infested and non-infested soils of the two moisture contents. It may be stated here that in seed-treatment experiments involving some thousands of seeds planted in outdoor plots, it had been observed in two successive years that the majority of seedlings emerged from the soil from May 9 to 16, during which period the soil temperature, based on the daily mean at one inch below the surface, averaged 15.4° C., in 1929, and 14.6° C., in 1930.

The tanks were operated at three different ranges of temperatures as follows:

Tank No. 1, 12–14° C., close to optimum for the host.

Tank No. 2, 18–20° C., close to optimum for the parasite.

Tank No. 3, 26–28° C., close to maximum for both host and parasite.

The number of seeds and roots involved in the experiment, together with other data and the results obtained are summarized in Table III.

TABLE III

RELATION OF SOIL MOISTURE AND TEMPERATURE TO ROT INFECTION OF GINSENG ROOTS AND SEEDLINGS

Seedlings

Tank	Temp., °C.	Container	M.H.C. of soil, %	Number of seeds planted		Seedling emergence		Healthy surviving seedlings	
				Sterile soil	Contam. soil	No.	%	No.	%
1	12–14	3	50	42	—	32	76	30	71
		4	50	—	42	8	19	3	7
		10	70	41	—	33	80	32	78
		12	70	—	42	6	14	3	7
2	18–20	2	50	41	—	3	7	3	7
		1	50	—	42	1	2	—	—
		8	70	40	—	4	10	4	10
		11	70	—	42	3	7	—	—
3	26–28	5	50	40	—	—	—	—	—
		6	50	—	42	—	—	—	—
		9	70	43	—	—	—	—	—
		7	70	—	42	—	—	—	—

Roots

Tank	Temp., °C.	Container	M.H.C. of soil, %	Number roots planted		Results		
				Sterile soil	Contam. soil	Healthy roots	Diseased roots	Per cent infection
1	12–14	6	50	4	—	4	—	Trace
		2	50	—	4	—	4	100
		12	70	4	—	4	—	Trace
		8	70	—	4	—	4	100
2	18–20	1	50	4	—	3	1	25
		4	50	—	4	—	4	100
		11	70	4	—	4	—	Trace
		7	70	—	4	—	4	100
3	26–28	5	50	4	—	4	—	—
		3	50	—	4	4	—	—
		10	70	4	—	4	—	—
		9	70	—	4	4	—	—

In Table III, it will be observed that in the 12–14° C., 50% M.H.C., sterile soil, 32 seedlings emerged, 30 of which, representing 71% of the number of seeds planted in container 3, remained healthy throughout the experiment. In the 12–14° C., 70% M.H.C., sterile soil, 33 seedlings emerged, 32 of which, representing 78% of the number of seeds planted in container 10, remained healthy. Even for selected seed, the percentage which germinated was high in both cases, the results thus tending to confirm the observations already made in outdoor experiments in connection with seed germination, that a temperature of about 15° C. is most favorable for the germination of seeds and the development of seedlings. In the 12–14° C., 50% M.H.C., infested soil, eight seedlings emerged, only three of which, representing 7% of the number of seeds planted in container 4, remained healthy. The same small number survived in the 70% M.H.C., infested soil in container 12, thus yielding only six healthy seedlings from a total of 84 seeds planted. It is apparent from the results that *Ramularia P*, even at a point 5° C. below its optimum for vegetative growth, is still extremely pathogenic on ginseng seedlings growing under conditions favorable for their normal development. Since at the two higher ranges of temperature, few or no seedlings survived, the results do not permit of further analysis.

During the period February 19 to April 19, the eight roots planted in the 12–14° C. *sterile* soil of both 50 and 70% M.H.C. (containers 6 and 12), developed normal tops which, at the termination of the experiment, had grown to a height of nine inches. No aboveground parts appeared in any of the other containers. When the roots of these eight plants were examined, it was found that, in general, the main taproots had remained healthy, but, in a number of instances, the tips of some of the laterals were diseased and, in one case, a main taproot had two small lesions on it. In Table III, under "results", these roots are tabulated as healthy, but on account of the slightly diseased condition referred to above, they are also listed under "per cent infection" as showing a trace of infection.

The roots planted in the 12–14° C. *infested* soils of 50 and 70% M.H.C., respectively, were without exception typically rotted, practically the whole of their surfaces being covered with large confluent lesions. The perennial stems showed some evidence of bud development but apparently the disease had progressed with such rapidity as to preclude further growth. *Ramularia P* was re-isolated in 88% of the plantings from these roots.

Of the eight roots planted in the 18–20° C. *sterile* soil of both 50 and 70% M.H.C. (containers 1 and 11), seven remained healthy except for diseased tips of laterals in a number of instances. The other root was found to be badly rotted. The perennial stems of the seven healthy roots showed only slight bud development. In the *infested* soil of the corresponding temperature and moisture series (containers 4 and 7), all eight roots were so badly rotted that it was impossible to remove them intact from the soil. They were in a much more advanced stage of disintegration than those planted in the 12–14° C. infested soil. *Ramularia P* was re-isolated in 84% of the plantings from these roots.

The 16 roots planted in the sterile soil or the infested soil at 26–28° C., remained healthy, all of them showing, however, a general, yellowish discoloration, the direct result, most probably, of the long exposure to the high soil temperature.

The results obtained in the above experiment indicate that the effect due to 6 to 8° differences in soil temperature was much more apparent than that due to a 20% difference in moisture content of the soil. At 12–14° C., in *infested* soil, the disease was not nearly so severe as at 18–20° C., while at 26–28° C. typical rot did not occur. At 12–14° C., the host is near its optimum for growth, while the fungus, on the other hand, is approximately 6° below its optimum. There are, thus, two factors involved whose combined effect tends to modify the severity of the disease. At 18–20° C., the effect of temperature on the host is unfavorable to the extent that the plants cannot proceed beyond slight bud development. On the other hand, the same temperature is most favorable for the growth of the pathogen. There is involved, then, a set of conditions, additive in their effect, which favor optimum disease development with consequent rapid disintegration of the roots. A temperature of 26–28° C. completely inhibits the development of the pathogen, and no disease results. The results of this experiment carried out under carefully controlled conditions in the laboratory confirm those already obtained in the field, indicating that at least one of the pathogens involved is extremely virulent in its attack over a relatively wide range of soil moisture and temperature conditions. There is some indication both from experimental as well as from observational results, that of the two variables, soil moisture and soil temperature, the latter is the more important factor in the incidence of the disease.

Series B. Involving Organisms Isolated from Rusted Roots

In addition to the infection experiments described above, corresponding series were carried out under field and laboratory conditions, using pure cultures of organisms isolated from rusted roots. To date, no definitely positive results have been obtained, but in a laboratory experiment recently completed, in which healthy roots were injured artificially and inoculated with three of five forms of *Ramularia* more recently isolated from rusted roots, certain evidence suggested that these three organisms may bear a causal relation to the rust disease. In this particular experiment, roots inoculated on November 21, 1932, were planted in steam-sterilized soil and kept in a cool cellar. Upon examination on February 21, 1933, several of the inoculated roots showed in the region of injury a depression roofed over by the still intact periderm. The space between the latter and the depressed surface of the root was occupied by a loosely compacted mass of rust-colored tissue closely resembling that found in typical rust lesions and, like the latter, very easily detachable. Free-hand sections from the depth of the concavities showed periderm formation between the superficial diseased tissue and the underlying healthy tissue. In the similarly injured

check plants, the wounds remained clean and healthy and there was no evidence either of the depression or of the masses of diseased tissue which were present in the case of the inoculated roots, but a periderm had formed over the exposed surface of the old injury.

Although the cause of the rust has not been definitely established, it can hardly be questioned that it is a disease entirely distinct from the rot. The circumstantial evidence based on general symptomatology, pathological histology, geographic distribution, association of organisms and results of infection experiments, seems sufficiently convincing to justify the view that the two are different diseases.

Evidence for their occurrence in certain of the ginseng-producing centres of the United States is to be found in Zinssmeister's paper (25), though that author did not realize that he was in all probability dealing with two distinct diseases. He had received specimens of diseased roots from Wisconsin and from New York, all of which he refers to as rusted material. He regarded rusted roots as exhibiting two types of lesions. His description of the first type in which "the injury is merely superficial and is confined to a few layers of cells immediately beneath the epidermis" and where "in many cases the root seems to be able to prevent the further ravages of the disease by cutting off the tissue attacked by the formation of a centripetal cork cambium," is perfect so far as it goes, for the disease known in Ontario as the rust. His description of the second type of lesion which instead of being confined to the sub-epidermal cells, extends to other tissues and finally causes the complete disintegration of the root, is identical with the symptoms of the Ontario disappearing-rot. From the Wisconsin roots which were in an advanced stage of decay and from the New York specimens which showed "the early stages of the disease" two species of *Ramularia* were isolated, both of which, when inoculated into artificially injured but otherwise healthy ginseng roots, were capable of producing "the *deep* type of rot" only. Apparently the superficial type of lesion was never reproduced in any of the infection experiments, which would make it appear that Zinssmeister had succeeded in isolating rot-producing organisms only. His two text-figures of diseased roots, the one showing "a surface view of ginseng roots attacked by *rust*", the other, "*rusted* roots in longitudinal section showing the *deep* type of lesion" are good illustrations of roots affected with the disappearing-rot.

It would appear that in the diseased specimens Zinssmeister obtained from the two widely separated sources, both the rust and the rot were present, either on different or, possibly, in some cases, on the same roots. This would not be inconsistent with what has been observed in Ontario, for the two diseases have been found encroaching upon roots in the same beds in certain gardens. There seems little doubt that the serious losses which for years have been incurred in many of the ginseng-growing centres of the United States and which have been attributed to the rust disease, are due, as in Ontario, not to one but to two distinct diseases.

The Pathogens

Literature Review with Reference to Isolation of Ramularia from Soil and from Underground Parts of Plants

Since Unger in 1833 established the genus *Ramularia*, it has been steadily augmented by the addition of new members until Saccardo (8, volumes 1 to 25 inclusive), records approximately 450 species, which with few exceptions have been found in association with the aboveground parts of plants. There are comparatively few references in the literature to members of the genus having been isolated either directly from the soil or from the underground parts of plants.

According to Wollenweber (24) in 1913, who gave not only the complete taxonomic history of the genus but also a revised generic diagnosis, five species of *Ramularia*, *R. candida*, *R. magnusiana*, *R. eudidyma*, *R. macrospora* and *R. olida*, were up to that time known to have been isolated from different underground parts of plants in widely separated districts. Only one of these, *R. olida*, had been found exclusively in association with subterranean parts of plants, the other four having been isolated, in addition, from leaves, stems and fruits. As the result of cross-inoculation experiments involving these different species, Wollenweber states that, "Ramularien, die als Blattparasiten bekannt sind, rufen ausserdem Knollen—und Fruchtfäule hervor und finden sich ausser auf Blättern auf unterirdischen Organen und ferner im Erdboden und auf Mist vor"; also that "Die Gattung Ramularia enhält eine Reihe ubiquitischer Wundparasiten". Sherbakoff (9), described and named a new species, *R. solani*, which was isolated from diseased tissue of a potato tuber affected with a superficial dry rot. Zinssmeister's work (25), demonstrating that *R. panacicola* and *R. destructans* n. spp. cause the disease of the roots of American ginseng popularly known as rust, is probably the first instance in which it has been shown that members of the genus are parasitic on the roots of any host plant. During investigations of yellow-leaf disease of *Phormium tenax*, Waters and Atkinson (15) isolated *R. phormii* from the roots of affected plants, in the tissues of which it was fructifying. Williams *et al.* (22) record a *Ramularia* sp. in connection with a root rot of *Statice latifolia*. Berkeley (1) isolated *Ramularia* spp. from rusted ginseng roots and in a single instance a *Ramularia* sp. was obtained from rot-infested soil. In 1927, Gilman and Abbott (4) published a summary of the fungi of the soil, according to which *R. eudidyma* Wollenw., and *R. macrospora* Fred., had been isolated from soil in Europe, while *R. magnusiana* had been obtained from soil in Texas. Walker (14) reports having obtained isolants of *Ramularia* during several seasons from diseased strawberry roots. Nobles (6) obtained from virgin soil in the vicinity of Toronto, Ontario, five isolants of *Ramularia* which were identified only as far as the genus.

Taxonomy

All isolants of *Ramularia* encountered in the present investigation were identified as such on a basis of Wollenweber's diagnosis of the genus (24,

p. 220). Specific identification was attempted only in the case of the three forms *M*, *P*, and *R* which had been shown definitely to be causal agents of the disappearing-rot. The three forms were studied critically in pure culture and their morphological and cultural characters were carefully compared with those of the definitely identified species recorded in the literature as having been isolated either from the soil or from the underground parts of plants. In studies of spore septation, and also for photographic purposes, the spores were mounted directly in a weak, aqueous solution of ruthenium red, following the suggestion of Brown and Horne (3) who employed this technique with success in their studies in the genus *Fusarium*. Spore samples for measurement were taken 1 cm. from the centre of 14-day-old petri dish cultures, grown at room temperature on potato-dextrose agar (2½% dextrose), pH 5.5, careful studies of the three forms at different stages of their growth having shown that at about 14 days they reached a stage of maturity when the conidia were of most uniform and typical shape and size. Photomicrographs were obtained using the Vernon (11) set-up which was found very well adapted to studies of this kind.

Ramularia P was found to correspond so closely with Zinssmeister's (25) *R. panacicola* as to leave little doubt that the two pathogens are identical. Two thousand four hundred conidia of *Ramularia P* (Plate II, Fig. 1) were found to measure 2.1–7.1 × 5.2–33.9 μ. Zinssmeister's measurements for 4,000 conidia are given as 2.5–7.2 × 5.5–34.2 μ. With regard to septation of conidia there is the close agreement shown in Table IV. Close uniformity in other morphological characteristics, including color of mycelium, size and color of chlamydospores and thickness and consistency of stroma in agar cultures, further confirm the identity of the two organisms.

TABLE IV

COMPARISON OF ZINSSMEISTER'S *R. panacicola* WITH *Ramularia P*

Organism	Number of conidia examined	Septation		
		0	1	2
Ramularia P	3000	69.6%	30.3%	.06%
R. panacicola	8000	69.0%	31.0%	—

Ramularia M cannot be identified with any of the species recorded in the literature as having been isolated either from soil or from the subterranean parts of plants. Two thousand conidia were found to measure 2.1–5.1 × 4.6–50.8 μ, 85% ranging in length from 5.0–19.0 μ. Whereas the larger conidia of *R. panacicola* (Plate II, Fig. 1) tend to be slightly pyriform, those of *Ramularia M* (Plate II, Fig. 2) are, in general, longer and more narrowly cylindrical. They are abstricted successively at the apices of simple, sparingly branched, or verticillately branched conidiophores, smaller spores being cut off first, followed by larger ones from the same conidiophores (Plate II, Fig. 6). Of 5,770 conidia examined as to septation, 83.6% were found to be 0-septate, the remaining 16.4% being 1-septate. The chlamydo-

spores of the two organisms are, in general, indistinguishable from one another and show wide variation in shape and size. Plate II, Fig. 5, shows an extreme in variation from the more constantly occurring type shown in Plate II, Fig. 4. Young cultures of the two organisms on potato-dextrose

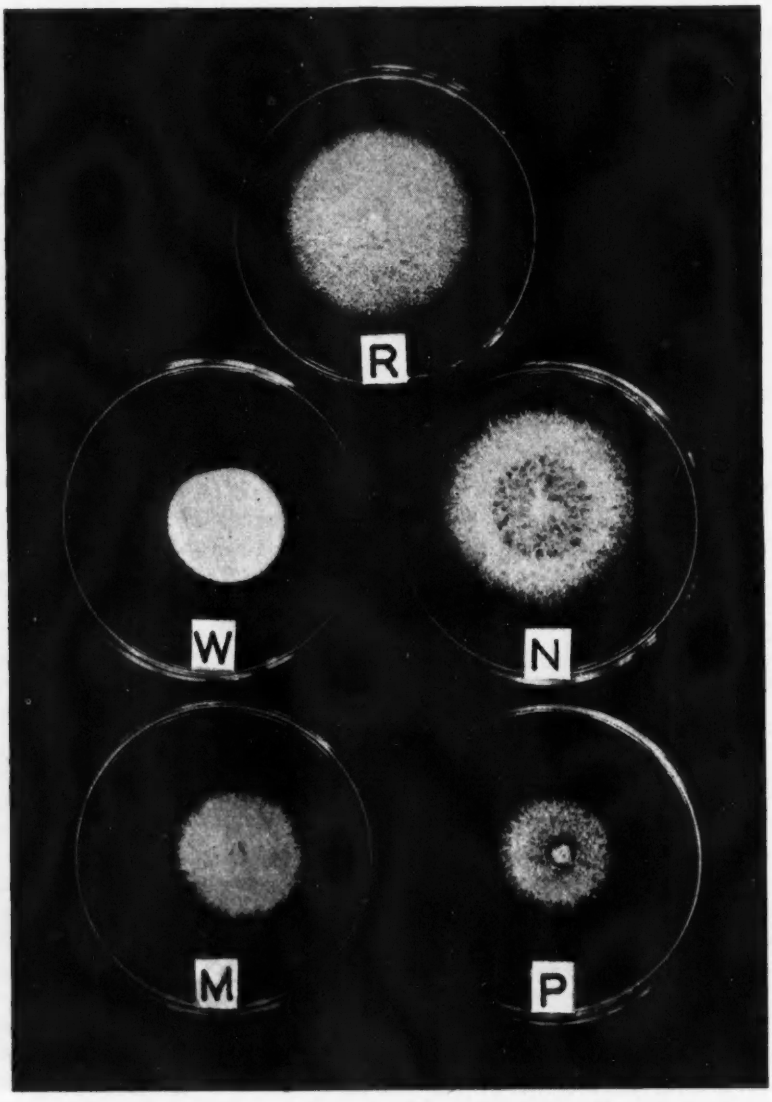
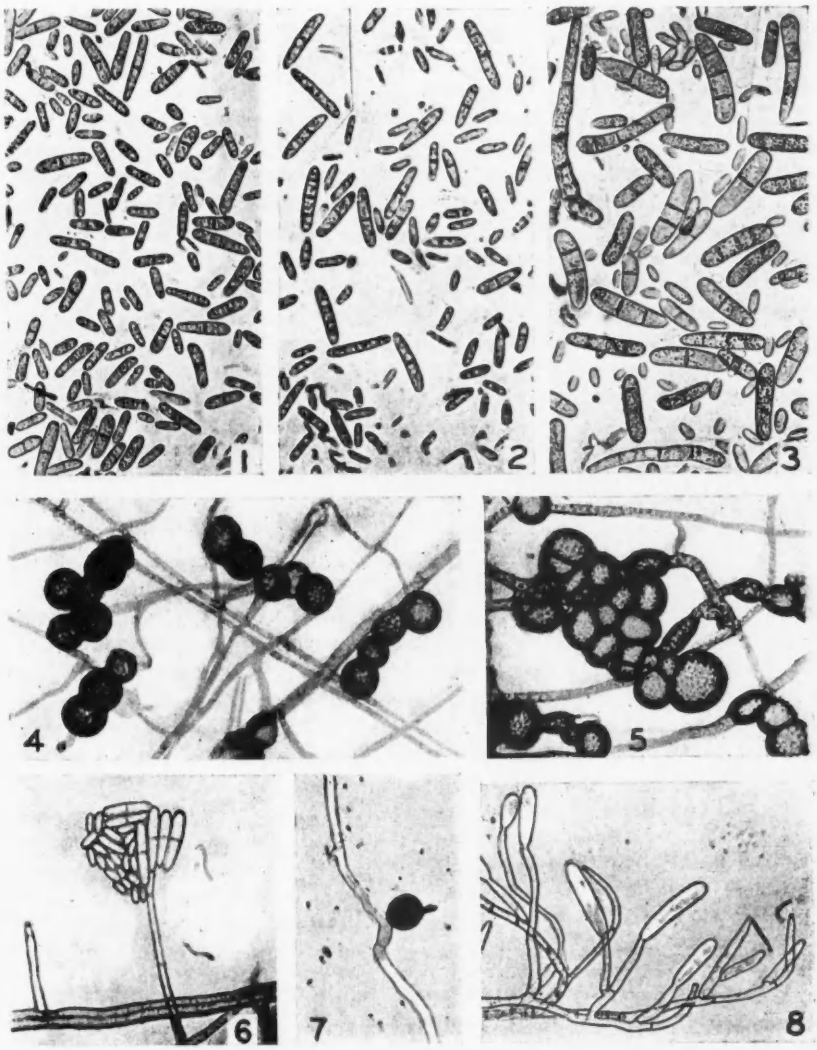


FIG. 2. *Ten-day-old cultures of R. robusta (R), R. panacicola (P), R. mors-panacis (M) from ginseng, Ramularia W (W) from strawberry and Ramularia N (N) from soil, incubated at 16° C., on potato-dextrose agar.*



Photomicrographs of three species of Ramularia causing disappearing-rot of ginseng. FIGS. 1-3. *Conidia of R. panacicola Zins., R. mors-panacis sp. nov., and R. robusta sp. nov., respectively, all from 14-day-old cultures grown on potato-dextrose agar (2½% dextrose), pH 5.5, at room temperature.* FIGS. 4 and 5. *Chlamydospores of R. panacicola and R. mors-panacis, respectively.* FIG. 6. *Simple type of conidiophore of R. mors-panacis. Note gradation in size of conidia and order of their production.* FIG. 7. *Germinating chlamydospore of R. mors-panacis.* FIG. 8. *Conidiophores of R. robusta. All* × *350.*

agar (2½% dextrose) closely resemble one another, but whereas the periphery of the colony of *R. panacicola* is always regular and sharply defined (Text-fig. 2, P) that of the colony of *Ramularia M* tends to be more or less irregular and indefinite, the hyphae of the latter organism merging almost imperceptibly into the substratum (Text-fig. 2, M). Young cultures of the two organisms are more readily distinguishable when grown on a starch-containing medium prepared according to the direction of Paul (7). *Ramularia M* like *R. panacicola* forms a thick leathery stroma on various agar substrata. In addition to the greater range in size of conidia, the higher percentage of non-septate spores and the less sharply defined edge of colony in agar culture, there are also certain other morphological and physiological characters in which *Ramularia M* differs from *R. panacicola*, sufficiently, it is believed, to be regarded as a distinct, new species for which the binomial *Ramularia mors-panacis* is proposed and which is described as follows:

Ramularia Mors-panacis sp. nov.

Technical Description

Conidia, hyaline; first-formed conidia smaller, ovoid to short cylindrical with ellipsoidal ends; later-formed conidia larger, fusiform or long narrowly cylindrical (Plate II, Fig. 6), infrequently papillate, not constricted at the septum; 0- to 1-septate, 84% of 5770 conidia, 0-septate, not producing chlamydospores endogenously; anastomosis of germ tubes rare; 2.1–5.1 × 4.6–50.8 μ, 85% of 2000 conidia ranging in length from 5.0 to 19.0 μ; conidia abstricted successively at the apices of simple, sparingly branched, or verticillately branched, septate conidiophores which may be scattered on the aerial mycelium or in sporodochia arising from the stroma; sterigmata more or less tapering, single or in groups up to four; aerial mycelium (on potato-dextrose agar (2½% dextrose), pH 5.5) abundant, septate, branched, at first white, loosely compacted, later showing transition through old-gold (R)* to Dresden-brown (R), two to several hyphal strands commonly aggregating to form coremium-like wefts; periphery of colony often irregular and indefinite; stroma thick, leathery, darker than claret-brown (R); chlamydospores abundant, intercalary, in chains or pseudo-sclerotial masses, darker than chestnut (R), irregular to spherical, the latter 7.8–23.4 μ (300 measured) in diameter; ascigerous stage not known.

Habitat in living roots of *Panax quinquefolium* L., Ontario, Canada.

*Ramularia Mors-panacis, sp. nov.***

Conidiis hyalinis, aliis primum formatis, minoribus, ovoideis vel breviter cylindraceis; deinde aliis majoribus, fusoideis vel longis et anguste cylindraceis, raro papillatis, in septo non constrictis; omnibus 0–1 septatis sed 84 per centum 0-septatis (5770 conidiis probatis), chlamydosporis non intra natis praeditis, 2.1–5.1 × 4.6–50.8 μ, quorum duorum milium 1700 conidia 5.0 usque ad 19.0 μ sunt longa, ab apicibus simplicium et raro vel longitudi-

**The symbol (R) is used here and subsequently to denote that the color nomenclature is that of Ridgway. Ridgway, Robert. Color standards and color nomenclature. 1912.*

***Latin diagnoses in this paper by Miss M. H. Thomson, 586 Spadina Avenue, Toront*

naliter ramificatarum conidiophorarum quae sparsa vel in sporodochiis posita sunt aliis post alios abruptis; sterigmatibus singulatim vel usque ad quattuor gragatim positis; mycelio aerio, in agar cultura innato, copioso, septato, ramoso, primo albo, deinde old-gold (R) vel dresden-brown (R), hyphis duabus vel pluribus fere innexis atque texturas formantibus; coloniis perimetro saepe irregulari indefinitaque praeditis; stromatibus crassis, lentis, obscurioribus quam claret-brown (R), chlamydosporis copiosis, intercalaribus, concatenatis vel in falsis sclerotiis positis, atrioribus quam chestnut (R), aliis irregularibus, aliis globosis, his 7.8–23.4 μ diam., evolutione sexuali ignota.

Hab., radicibus vivis *Panacis quinquefolii* L., Ontario, Canada.

Ramularia R like *R. mors-panacis* cannot be identified with any of the species recorded as having been isolated from the soil or from the underground parts of plants. This organism is more conspicuously different from either *R. panacicola* or *R. mors-panacis* than are the latter from each other. The conidia (Plate II, Fig. 3) are not only much larger but are also more variable in shape and may have as many as eight septa. Five thousand six hundred and twenty conidia were found to range in size from 1.4–9.1 × 3.1–122.0 μ. Approximately 98% of the conidia are less than 65.0 μ in length and do not possess more than three septa but included in the remaining 2% are "giant" conidia, the largest observed measuring 122.0 μ in length. In agar cultures smaller conidia are abstracted successively at the tips of simple to sparingly branched conidiophores (Plate II, Fig. 8) arising from the aerial mycelium which, as in the case of both *R. panacicola* an *R. mors-panacis* consists either of individual hyphae or of strands of the latter aggregated into coremium-like wefts. The smaller conidia often adhere to the tips of the conidiophores in small gelatinous heads. Larger conidia occur most profusely as pionnotes on the surface of various agar substrata. On the latter, *Ramularia R* forms a thin stroma and, in old or desiccated cultures, chlamydospores, brown in color and mostly intercalary in position, are produced but much less abundantly than in the case of the other two ginseng pathogens. *Ramularia R* grows much more rapidly at room temperature than either *R. panacicola* or *R. mors-panacis*. *Ramularia R*, in addition to its conspicuously larger conidia, its thinner stroma and relative paucity of chlamydospores together with its faster growth rate, exhibits other morphological, physiological and pathological characteristics which mark it as so distinctly different from *R. panacicola* and *R. mors-panacis* as to warrant regarding it as a distinct, new species for which the binomial *Ramularia robusta* is proposed and which is described as follows.

Ramularia Robusta sp. nov.

Technical Description

Conidia hyaline; smaller conidia ovoid to broadly cylindrical, abstricted at the apices of simple to sparingly-branched conidiophores arising from the mycelium, often adhering to the conidiophores in small gelatinous heads; larger conidia broadly cylindrical, clavate, mostly slightly curved, infrequently papillate, not forming chlamydospores endogenously and not constricted at

the septa but giant conidia often variously curved and distorted, occurring most profusely as pionnotes covering surface of stroma; 0–8-septate; 1.4–9.1 × 3.1–122.0 μ (5620 conidia examined as to size and septation); 33%, 1-septate, 4.0–8.2 × 10.5–37.4 μ, average 5.9 × 21.6 μ; 4.8%, 2-septate, 5.4–8.4 × 24.5–38.6 μ, average 6.9 × 30.8 μ; 5.2% 3-septate, 5.6–9.1 × 27.6–65.3, average 8.2 × 42.5 μ; aerial mycelium (on potato-dextrose agar (2½% dextrose), pH 5.5) septate, branched, at first white, soon becoming buffy-brown (R), sparse, later merging with the substratum and giving the surface a mealy appearance; anastomosis of hyphae frequent and two to several strands aggregating to form coremium-like wefts; stroma, thin, seal-brown (R); chlamydospores in old or desiccated cultures mostly intercalary, in chains or pseudo-sclerotial masses, buffy-brown (R), mostly irregular, at times spherical, the latter up to 14.5 μ in diameter; ascigerous stage not known.

Habitat in living roots of *Panax quinquefolium* L., Ontario, Canada.

Ramularia Robusta sp. nov.

Conidiis hyalinis, aliis minoribus ovatis vel late cylindraceis, ab apicibus simplicium vel parce ramosarum et in mycelio sparsarum conidiophorarum abruptis, saepe in eis parvis gelatinosis globis adhaerentibus; aliis majoribus, creberrime quam pionnatibus, late cylindraceis, clavatis, fere paulo flexis, infrequenter papillatis, chlamydosporas non intra formantibus neque ad septa constrictis sed maximis saepe varie curvatis et detortis; omnibus 0–8-septatis, 55 per centum 0-septatis, 33 per centum 1-septatis, 5 per centum 2-septatis, 5 per centum 3-septatis; 1.4–9.1 × 3.1–122.0 μ, quorum 5620 omnium 5508 conidia non longiora sunt quam 65.0 μ; mycelio aerio, in agar cultura alito, septato, ramoso, primo albo, mox buffy-brown (R), sparso, demum substrato conjuncto hoc et superficies granulosa videatur efficiente; anastomosibus inter hyphas frequentibus, et duabus vel pluribus hyphis fere innexis atque texturas formantibus; coloniis perimetro regulari et definita instructis; stromatibus tenuibus, seal-brown (R); chlamydosporis in veteribus vel desiccatis culturis crescentibus, plerumque intercalaribus, concatenatis vel in falsis sclerotiis positis, buffy-brown (R), multis irregularibus, sed paucis globosis, his usque ad 14.5 μ diam.; eis que ad generationem pertinent ignotis.

Hab., in radicibus *Panacis quinquefolii* L., Ontario, Canada.

To date the three species have remained remarkably uniform in culture. After a long series of transfers the conidia and chlamydospores show no diminution in quantity or size. A sexual stage has never been observed and apparently cannot be induced either by subjecting the three organisms to varying environmental conditions or by growing them together in culture.

Spore Germination

Conidia of the three species will readily germinate in two hours in tap water at 20° C. In 24 hours at room temperature, chlamydospores will germinate (Plate II, Fig. 7) as readily as conidia in drops of tap water. Non-septate spores show the same facility for germination as those which possess septa.

Relation of Temperature to Growth in Pure Culture

The three species from ginseng, an isolant from diseased strawberry roots, supplied by A. R. Walker (14), and one of Miss Nobles' (6) isolants from virgin soil were grown under carefully controlled environmental conditions, in petri dishes on potato-dextrose agar (2½% dextrose), at 2° C. intervals over a temperature range of 0–30° C. At each temperature interval, each of the five isolants was grown in triplicate, thus totalling fifteen plates per series. Data were recorded for two complete and independent series involving approximately 450 plate cultures. The cultures were incubated for ten days at each temperature interval, at the end of which time maximum radial growth was determined on the basis of diameter of colony.

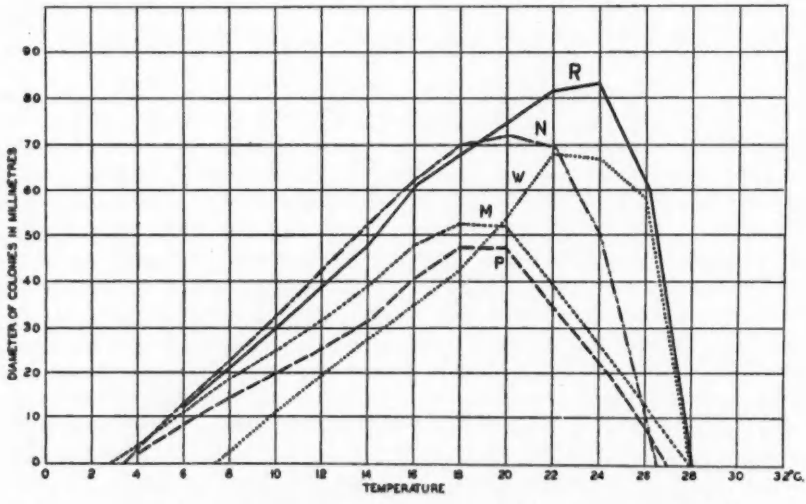


FIG. 3. *Curves showing relative growth of R. panacicola (P), R. mors-panacis (M), R. robusta (R) from ginseng, Ramularia W (W) from strawberry, and Ramularia N (N) from soil, as indicated by diameter of colonies of the five organisms, after incubation at 2-degree intervals for 10 days on potato dextrose agar.*

The three species from ginseng have practically the same growth range, from about 3 to 28° C. (Text-fig. 3). The optimum for *R. mors-panacis* and *R. panacicola* occurs about 18–20° C., while that of *R. robusta* is nearer 24° C. The latter, throughout its range, exhibits more abundant growth than the other isolants, with the exception of *N*, the isolant from soil. *R. mors-panacis* and *R. panacicola*, which closely resemble one another in their pathogenicity, exhibit the same general temperature requirements upon artificial media, their growth curves showing a close parallelism, with *R. panacicola* lagging slightly behind *R. mors-panacis*. The isolant from strawberry roots, *W*, with its minimum for growth at about 7° C. and its optimum from 22 to 24° C., exhibits higher temperature requirements than *R. mors-panacis* and *R. panacicola* from ginseng.

Longevity in the Soil

At various times during the course of the present investigations attempts were made to isolate the pathogens from soil samples taken from plots where the disease was definitely known to have been especially severe. Soil dilutions varying in concentration from strong to weak (1 : 500 to 1 : 1,000,000) were mixed with, or applied to, the surface of various media which are reported as having been used with success by other workers investigating fungi of the soil (4, 6, 13, 16). Plates were incubated over a range of temperatures which included the optima for vegetative growth of the three rot-producing organisms. To date, neither the latter nor any other representative of the genus *Ramularia* ever appeared in any of over 400 poured plates. It remains impossible, therefore, to state definitely how long or in what form the pathogens may persist in the soil but evidence from field observations indicates that they remain viable for considerable lengths of time. The numerous failures to produce second crops of ginseng in soil that has once become infested and the increasing number of gardens abandoned because of ginseng-sick soil both point to the ability of the pathogens to live over and accumulate in the soil.

Cross Inoculation Experiments

The occurrence of individual rotted roots variously scattered throughout an otherwise healthy stand of ginseng is difficult to account for. The only possible explanation in certain cases would seem to be the presence of a pathogen residual in the soil. Miss Nobles' (6) discovery of representatives of the genus *Ramularia* residual in virgin soil in Ontario seemed particularly significant. If it could be shown that a *Ramularia* residual in the soil was pathogenic on ginseng, this might explain the occurrence of disease in new stands grown from treated seed. Especially interesting in this connection is the following excerpt from Werkenthin (16) who, in 1916, investigated the fungous flora of Texas soils, "Of special interest in the study of soil fungi is the fact that the virgin soil contained fungi which are known to be parasitic to cultivated plants, *e.g.*, *F. solani*, *F. oxysporium* and *F. radicicola*. It also should be noted that these fungi were isolated several times from the same plot during a period of over five months, which fact should show clearly that these fungi are true inhabitants of the soil". Of interest, too, in connection with the present investigations was Walker's (14) assigning the cause of strawberry root rot in the Niagara Peninsula to a *Ramularia* sp. In the Waterford district, strawberry root rot is becoming as serious a problem as ginseng root rot and in many instances ginseng and strawberries are grown in close proximity to one another. In view of the possibilities suggested by the above findings, the following series of pathogenicity tests were carried out.

Series 1. Pathogenicity of Isolants of *Ramularia* from Strawberry and from Soil, on Ginseng

Healthy, two-year-old roots, were immersed for 8–10 min. in 1 : 1000 bichloride of mercury, then rinsed through three changes of sterile water, and artificially injured by making an incision $\frac{1}{4}$ to $\frac{3}{8}$ in. long, using a flamed scalpel. Inoculum from 14-day-old tube cultures of forms *W* (strawberry), *N* (soil), and the three species from ginseng was inserted into the incision. Check roots were similarly injured but not inoculated. The roots were planted in sterile soil in pots which were left in a cool cellar for 36 days, sterile water being added as required. Sixty roots were involved in the experiment, 10 serving as checks and 10 being inoculated with each of the five isolants of *Ramularia*.

On all 10 roots inoculated with *Ramularia W*, small necrotic areas developed in the immediate region of the injury (Plate III, Fig. 1, W). The organism was recovered in 90% of the plantings from necrotic tissue.

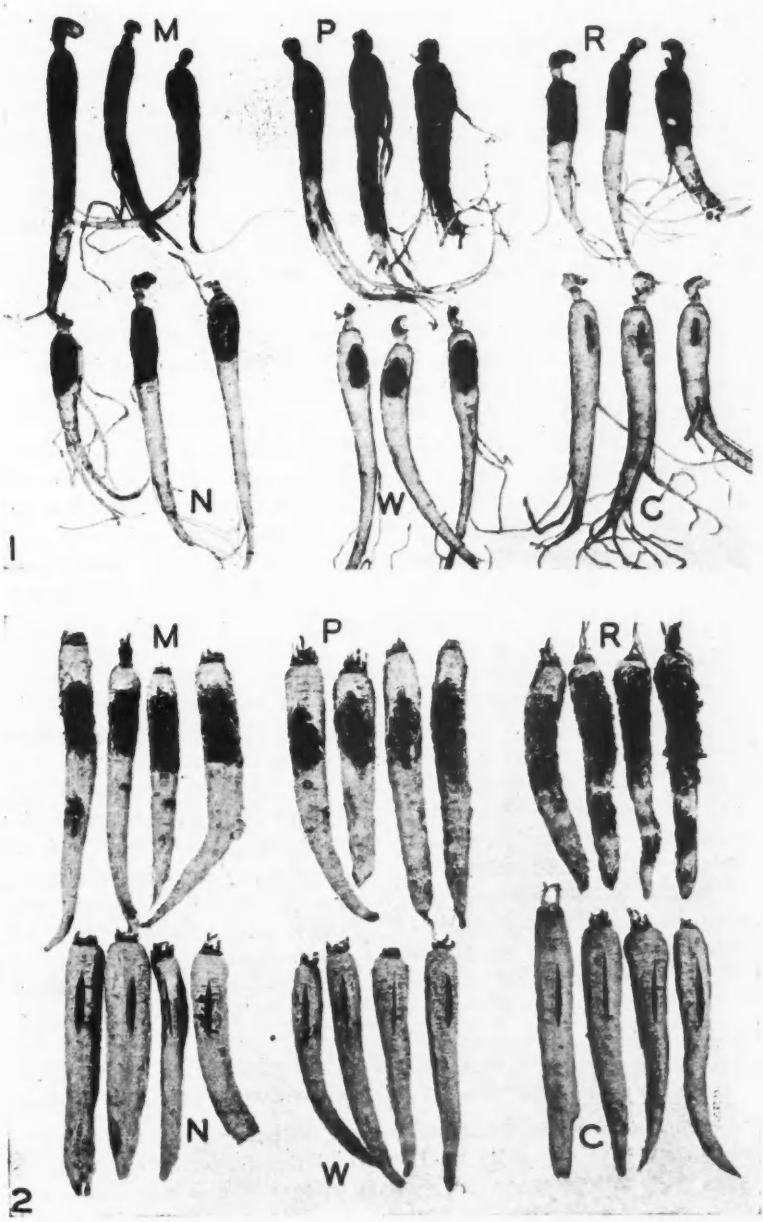
On all 10 roots inoculated with *Ramularia N*, definite lesions developed in the region of injury (Plate III, Fig. 1, N). The lesions, however, were not typical of those of the disappearing-rot (Plate III, Fig. 1, M, P and R), being more sharply delimited both externally and internally from healthy tissue and, in color, a light, yellowish brown rather than dark, reddish brown. The organism was re-isolated from 87% of the plantings from diseased tissue.

The check roots all remained healthy (Plate III, Fig. 1, C). Results obtained earlier with the isolants from ginseng were confirmed.

The results recorded above, which were confirmed in subsequent tests, demonstrate that the five isolants of *Ramularia* possess different infection capabilities where ginseng is concerned. On this host, the isolant from strawberry is only slightly pathogenic, the soil organism exhibits a somewhat greater degree of virulence, and, as has been demonstrated consistently, the other three isolants are aggressive parasites, *R. robusta*, however, not displaying the same degree of virulence as the other two species (Plate III, Fig, 1, M, P and R).

Series 2. Pathogenicity of Isolants of *Ramularia* from Ginseng and from Soil, on Strawberries

On July 22, 1931, strawberry plants, variety Glen Mary, grown for 42 days in the greenhouse from runners trained over sterile soil in 5-in. pots were transferred to 9-in. pots containing soil which had first been sterilized and then infested with *R. panacicola* and *R. mors-panacis* (ginseng), and *Ramularia N* (soil), growing on sterilized oats. Thirty-two plants were involved in the experiment, three lots of eight each being transferred to soil infested with *R. panacicola*, *R. mors-panacis* and *Ramularia N*, respectively, while the remaining eight plants in non-infested soil served as checks. The 9-in. pots were transferred to outdoor plots where they were sunk until their tops were about two inches above the level of the surrounding soil. Through-



FIGS. 1 and 2. *Showing variations in pathogenicity and specificity in host relations among isolants of Ramularia.* FIG. 1. *Ginseng roots artificially infected with R. morspanacis sp. nov. (M), R. panacicola Zins. (P), R. robusta sp. nov. (R), from ginseng, Ramularia N (N) from soil and Ramularia W (W) from strawberry, with check roots (C).* FIG. 2. *Carrots infected with same organisms as above.*

out the remainder of the 1931 growing season no difference could be noted between plants growing in infested and non-infested soil. Further observations were not possible until May 25, 1932.

On that date, a striking difference was noted between the plants grown in the soil to which *Ramularia N* had been added and the remainder of the plants. Of the former, four had almost completely disappeared except for a few shrivelled remains of tops and roots, two were dying and, when removed from the soil, were found to have a very limited and badly diseased root system, and the remaining two plants, though still living, were "dwarf plants" with correspondingly limited root systems. From brownish, discolored areas on the roots of the stunted and dying plants, 60 tissue plantings were made and *Ramularia N* was recovered from 86% of the plantings.

One of the plants grown in the soil infested with *R. mors-panacis* was dead, apparently having died, however, before growth was resumed in the spring. The remaining seven plants of the series had well developed root systems which appeared to be perfectly healthy except for the occurrence on a few laterals of scattered lesions from which *R. mors-panacis* was recovered in a few instances.

In the case of plants grown in soil infested with *R. panacicola* the results obtained were almost identical with those described above for *R. mors-panacis* except that all of the plants of the series had remained healthy. From a few brownish, discolored areas on a number of the laterals, *R. panacicola* was recovered in pure culture.

One of the eight check plants was dead, apparently having died during the winter as there was no indication of current-year growth. The remaining seven plants had healthy, well developed tops and root systems.

From the results obtained in the above experiment and from the preceding one, it would appear that the presumably saprophytic isolant from the soil, *Ramularia N*, can, under certain conditions, at least, become parasitic on the roots of certain living plants. On strawberry roots the organism is much more aggressive in its attack than on ginseng roots. The results of this experiment also suggest a marked degree of specificity on the part of two of the species isolated from ginseng. These species which are such aggressive parasites on ginseng cannot attack healthy roots of the variety of strawberry involved in the experiment. This fact was confirmed later in greenhouse experiments.

Series 3. Pathogenicity of Isolants of *Ramularia* from Ginseng, from Strawberry and from Soil, on Carrots

Forty-eight healthy carrots were surface-sterilized for 8 to 10 min. in 1 : 1000 bichloride of mercury and then rinsed through three changes of sterile water. They were then apportioned at random into six groups of eight each. Four from each group were artificially injured by making an incision about ½ in. long, with a flamed scalpel. These narrow incisions were packed with inoculum obtained from 14-day-old cultures of the five isolants of *Ramularia*.

Injured checks were treated in identically the same manner using sterile agar. The four remaining carrots of each group were dipped in spore suspensions of each organism. All were planted June 8, 1932, in sterilized soil in pots which were transferred to the outdoor plots where they were left until July 11, sterile water being added as required.

Every carrot produced an abundant growth of green, healthy tops and a new root system, a response in marked contrast to the passive behavior of ginseng roots subjected to similar treatment. Towards the end of the experiment the tops of the plants which had been injured and inoculated with *R. robusta* showed signs of wilting. On July 11, when the roots were examined, it was found that of the roots which had been artificially injured only those which had been inoculated with the species of *Ramularia* isolated from ginseng were diseased (Plate III, Fig. 2, M, P and R). *R. robusta*, which on ginseng is always less virulent in its attack than *R. panacicola* or *R. mors-panacis*, displays the greatest degree of virulence on carrots, causing a much greater disintegration of root tissues in 33 days than either of the other species. All three species were recovered in a high percentage of cases from the respective roots into which they had been inoculated. All the roots injured and inoculated with isolants *N* and *W* remained healthy, as did the checks (Plate III, Fig. 2, N, W and C).

In the case of the carrots inoculated by immersion in spore suspensions of the five organisms, the results closely paralleled those described above. Only those roots which had been dipped in spore suspensions of the three isolants from ginseng became diseased, but in no instance were they as severely attacked as when injured and inoculated. Again *R. robusta* was the most virulent pathogen. All the carrots inoculated with isolants *N* and *W* remained healthy, as did the checks.

The results described above, which were confirmed in other experiments, suggest further the degree of specificity exhibited by representatives of the genus *Ramularia* obtained from different sources. The isolant from strawberry, which in a previous experiment was shown to be slightly pathogenic on the roots of ginseng, does not attack carrots even when an infection court is provided. The same is true for *Ramularia N*, the soil isolant, which, however, shows marked aggressiveness in its attack on the roots of strawberry and is also pathogenic to a degree on ginseng. *R. mors-panacis*, *R. panacicola* and *R. robusta* show marked aggressiveness in their attack both on ginseng and on carrots, but the two first-mentioned species when tested on strawberries did not attack that host.

Seed Treatment Experiments

Believing that the dissemination of disease is associated with the seed and that remedial measures are to be found in the disinfection of the seed, many of the growers in Ontario have adopted a method of seed treatment which involves immersion in a solution of formaldehyde. The effect of such treatment on the germination of the seeds, especially when the seed coat is already

cracked open, whether the diseases really are seed-borne and, if so, the effectiveness of the treatment in combating them, remained to be investigated.

In September 1930, 1200 seeds with the seed-coats cracked open were selected from a quantity of seeds which, having been picked the previous autumn, had passed through twelve of the eighteen months of their natural period of dormancy and should, therefore, germinate the following spring. They were apportioned at random into four lots of 300 each. Lot A was suspended in sterile water for one hour; lot B was immersed in a solution of formaldehyde (2 oz. per gal.) for one hour; lot C was soaked for one hour in a heavy spore suspension of *R. panacicola*, in which both conidia and chlamydospores were abundant; lot D, after soaking in the same spore suspension for one hour, was immersed in the formaldehyde solution (2 oz. per gal.) for one hour. The strength of solution and the period of immersion were chosen as being most closely in accordance with the treatments most generally in use in the Waterford district. Without being allowed to dry, the seeds were planted, Sept. 28, 1930, in an outdoor plot, the soil of which was covered with a mulch of leaves.

The numbers of seeds involved, the treatments to which they were subjected and the resultant percentage germinations as evidenced by seedling emergence May 4 to May 23, 1931, are summarized in Table V.

TABLE V

EFFECT OF FORMALDEHYDE TREATMENT ON GERMINATION OF GINSENG SEEDS AND ON VIABILITY OF TYPICAL SPORE FORMS OF *R. panacicola*

No. seeds planted	Treatment	Germination %
300	One hour in sterile water	84.6
300	One hour in 2-ounces-to-the-gallon formaldehyde solution	88.4
300	One hour in spore suspension of *R. panacicola*	8.7
300	One hour in spore suspension of *R. panacicola* followed by one hour in 2-ounces-to-the-gallon formaldehyde solution	77.4

From the results of the experiment as recorded in Table V, it would appear that soaking seeds, even when cracked open, for one hour, in a solution of formaldehyde (2 oz. per gal.), rather than having an adverse effect, tends to stimulate their germination, also, that such treatment is most effective in destroying the viability of conidia and chlamydospores of *R. panacicola* present on the surface of the seeds.

An experiment similar to the one described above but involving about 3600 seeds was performed in the fall of 1931 and the results obtained in the 1930-31 experiment were closely confirmed. Again it was noted that the formalin-treated seeds showed about 4% increase in germination over those treated with sterile water alone.

Having adduced some evidence that the formaldehyde treatment, as generally employed, would be effective in controlling the rot if it were a case of the typical spore forms of the pathogen being carried mechanically on the surface of the seed, there still remained the possibility of failure of such treatment to control the disease if the parasite were localized within the seed coat or in the reproductive organs of the seed. Mature "berries" were collected from plants whose roots were typically rotted. Material including the fleshy mesocarp and the endocarp with its contents, was fixed, sectioned, stained and examined microscopically. No evidence of the presence of a pathogen in any of the tissues was found in the material examined. Other "berries" were surface sterilized and tissue plantings from the mesocarp were made on potato-dextrose agar. In other cases, following surface sterilization of the epicarp and the removal of the mesocarp aseptically, the endocarp with its contents was transferred, in whole or in part, to the same nutrient medium. In many series of such plantings, the rot-producing organisms never appeared in culture.

In addition to the above studies, many sections from various regions of the stems of plants with diseased roots have been examined microscopically, and plantings have been made to various nutrient media. In not a single instance has it been possible to demonstrate the presence of the pathogens in any part of the stem above the ground level. All the evidence, both from laboratory experiments and from observations in the field, points to the fact that the rust and the rot are not systemic diseases but are confined to the parts of the plant that remain underground.

While it is believed that neither the rust nor the rot is transmitted through the seed and that ordinarily the pathogens are not present on the surface of the seeds, it is not considered impossible that at some stage in their handling they may become contaminated. Not only as a safeguard against the latter contingency but also as a possible important factor in the control of another important disease of ginseng, the leaf and stem blight caused by *Alternaria panax* Whetzel, the spores of which fungus have been found in abundance on the surface of a large number of seeds examined in the present investigations, it is recommended that all seeds, preferably just prior to planting, be immersed for a period of one hour in a solution of formaldehyde (2 oz. per gal.), care being taken to plant the seeds before they become dry. Such treatment has been found to increase germination to a slight but appreciable extent (about 4%).

Control

With either or both of the diseases already firmly established in the principal ginseng-growing districts of Ontario, exclusionary or eradicatory measures for their control offer very limited possibilities. Brann (2) reported that results obtained in Wisconsin following steam sterilization of soil by the inverted-pan method indicated that the process was at least partially effective in controlling the brown root rot, or rust, of ginseng. Under existing economic

conditions sterilization of soil by heat is as impracticable to the majority of the ginseng growers in Ontario as is soil disinfection by the application of chemicals, except where relatively small areas are involved. With a view to obtaining information as to the possibility of effectually preventing the spread or occurrence of the diseases by the application of disinfectants, a series of experiments involving the use of formaldehyde, Bordeaux mixture, copper sulphate solution, bichloride of mercury, sulphur and paradichlorobenzene, on small areas of either artificially or naturally infested soils have been carried out in laboratory experimental plots and in the field at Waterford. The results in general have been of such a contradictory nature that it remains impossible to recommend with certainty the use of any of the above-mentioned chemicals for the effectual control of either the rust or the rot when they make their appearance in a stand of ginseng.

In the hope of securing resistant varieties certain of the growers at Waterford have visited gardens in the United States and from those which appeared to be free from disease have secured seeds and young roots for transplanting. The general experience has been that imported stock has proved to be as susceptible to rust and rot as native stock. Nor does the possibility of securing disease-resistant plants by selection seem to offer much hope as a factor in control. During the past four years it has been observed that when the rot spreads from a centre of infection almost invariably every plant succumbs. Even if certain plants did survive and seeds were obtained from them, the task of developing a disease-resistant strain would be a particularly long and arduous one. Ginseng seeds do not germinate for 18 months from the time they are picked and plants do not produce their first crop of seeds until the third growing season.

Discussion

In the course of the present investigations ten representatives of the genus *Ramularia* have been found more or less consistently in association with a diseased condition of ginseng roots. Five of them were isolated from typically rusted roots and five from characteristically rotted roots. Three of the five isolants from the latter source, which have been shown to bear a primary causal relation to the rot disease, have been studied intensively in an attempt to identify them with the relatively few species of the genus reported as having been isolated either directly from the soil or from the underground parts of plants. No effort was made, however, to establish their identity with any of the large number of species (approximately 450) which have been found in association with the aboveground parts of plants. One of the pathogens, referred to in the body of the paper as *Ramularia P*, was identified as *Ramularia panacicola* Zins., but the other two, designated as *Ramularia M* and *Ramularia R*, could not be identified. Pathologically, *Ramularia M* closely resembles *R. panacicola* but differs from the latter in certain morphological, cultural and physiological characteristics. *Ramularia R* differs distinctly from the other two pathogens, morphologically, pathologically and

physiologically. The evidence which has been presented is regarded as sufficient to warrant the description of *Ramularia M* and *Ramularia R* as two new species for which are proposed the binomials *R. mors-panacis* and *R. robusta*.

The present investigations have also given some indications of the parasitic capabilities of at least five different representatives of the genus. Cross-inoculation experiments involving unrelated hosts and isolants of the fungus obtained from entirely different sources have indicated variations in pathogenicity among the isolants as well as specificity in host relationships. An isolant from virgin soil (*Ramularia N*) has been shown to be an aggressive parasite on the roots of strawberry. The same organism is pathogenic to a degree on the roots of ginseng but possesses no capacity for attacking the roots of carrots even when an artificial infection court is provided. Three isolants from ginseng, *R. panacicola* Zins., *R. mors-panacis* n. sp., and *R. robusta* n. sp., all aggressive parasites on that host, exhibit marked differences in their virulence, the two first-named species being capable of bringing about much more rapid disintegration of the host than *R. robusta*. The latter species, however, is much more virulent in its attack on carrots than the other two ginseng pathogens. An isolant from strawberry (*Ramularia W*) is slightly pathogenic on the roots of ginseng but cannot attack the roots of carrot even when the internal tissues have been exposed by injury. It may be mentioned here that, incidental to the present studies, ten additional representatives of the genus *Ramularia*, different from each other and from the ten isolants obtained from ginseng, have been isolated from definite and characteristic lesions occurring upon otherwise healthy, functional tissue of (i) the rootlets of apple, (ii) the suckers of raspberry, and (iii) the finer lateral roots of both strawberry and *Agropyron repens*. An additional isolant has also been obtained from the older roots of grape, showing discoloration of vascular tissue. In the light of the above it would appear that the genus *Ramularia* must be regarded as an important member of the group of facultative parasites associated with root troubles of plants.

An interesting feature of the present work is the demonstration that representatives of the genus *Ramularia* occur more abundantly in association with the underground parts of plants than the literature to date would indicate. If a concerted effort were made to examine the subterranean parts of a still wider range of possible host plants, it is believed that this fungus would be shown to occur as ubiquitously in a soil environment as it does on aerial parts of plants. Why it has not been reported more frequently by other workers investigating root troubles of plants is difficult to understand. One reason may be that it has been overlooked. The members of the genus isolated in the present work grow, in general, much more slowly at room temperature than the representatives of several of the genera so frequently encountered in studies of this kind, including *Fusarium*, *Penicillium* and members of the *Mucoraceae*. Unless mixed cultures developing from tissue plantings are examined most carefully, the presence of *Ramularia* can easily

be overlooked. Tube cultures in particular, because of the early appearance of fungi regarded as contaminants, are no doubt discarded in many instances before the presence of *Ramularia* can be detected. Another reason why the organism has not been reported more frequently may be that it has been mistaken for other fungi. Certain members of the genus are, morphologically, quite similar to border-line representatives of other genera, for example, *Fusarium* and *Cylindrocladium*. Under the circumstances it becomes difficult for investigators who are not thoroughly familiar with these groups to distinguish the representatives of one genus from those of another.

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A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

I. PREPARATION, TOXICITY AND BIOCHEMICAL NATURE OF ALCOHOLIC PRECIPITATE[1]

By Ronald Gwatkin[2]

Abstract

Alcoholic precipitates were prepared from shaken and filtered suspensions of *Brucella abortus*. These precipitates were highly toxic for guinea pigs by intraperitoneal injection. The toxic and antigenic fraction was water soluble. Filtration did not modify the toxic effect. Dialysis removed some of the lethal substance in one trial but did not affect the suspension in a subsequent experiment. Varying volumes of alcohol did not affect the toxic and antigenic qualities of the precipitate. Preparation of these precipitates on several occasions gave rise to symptoms simulating undulant fever in a hypersensitive human subject. Preliminary biochemical examination suggested that the precipitate consisted almost entirely of carbohydrates.

Introduction

Rake (4, 5, 6, 7) has reported work on fractions of the meningococcus. One paper (6) deals with the precipitation by alcohol of a solution of ground meningococcus. This solution was filtered. One portion was precipitated with eight volumes of 95% alcohol dissolved in distilled water and reprecipitated until free from non-carbohydrate substance. This was not type nor species specific. Another portion, precipitated with 1½–2 volumes of alcohol and reprecipitated until free from non-carbohydrate substance was classified as the soluble or S-specific substance.

Zozaya (8) in 1931 reported a serological study of the polysaccharides of meningococcus, *B. anthracis*, *B. proteus*, *B. subtilis* and *B. mesentericus*. He found that the meningococcus polysaccharide reacted with a broad precipitable carbohydrate antibody in common with those of the other organisms. Agglutinins had no relation to the carbohydrate precipitable substance, specific or non-specific.

Przesmycki (3) in 1924 published a paper on specific "Residue Antigens" of different types of meningococci. These were prepared as follows: Blake bottles were washed off with 25 cc. of salt solution each. The suspension was shaken five to six hours. Gross particles were removed by centrifugation until an almost clear, opalescent, yellowish fluid was obtained. This was precipitated by the addition of 10% acetic acid drop by drop. Usually but little precipitate was obtained. This was removed by centrifugation. The supernatant fluid was boiled two to three minutes and the precipitate again removed. The reaction was adjusted to pH 7.0 with 10% sodium hydroxide, and to the fluid was added five volumes of absolute alcohol. It was left at room temperature 12 to 16 hr. The precipitate was collected by centrifugation, washed with alcohol and ether and then dried. A very small amount of a grayish-white precipitate was obtained. A suspension of 1 : 100 was made in distilled water by the addition of acetic acid, which was afterwards neutral-

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ized. Serums prepared by injections of meningococci showed that the residue antigens gave specific reactions with homologous serums. Heterologous serums gave little or no reaction.

Huston, Huddleson and Hershey (2) in 1934 published a study of the chemical separation of some cellular constituents of the *Brucella* group of micro-organisms.

The *Brucella* were found to be characterized as a group by the absence of free simple sugars, by the occurrence of non-precipitating polysaccharides only, by a large proportion of water-extractable proteins, and by cell lipides analogous to the conventional types found in higher organisms.

The species of the genus *Brucella* could be differentiated one from another by the relative proportions, rather than kind, of two biologically inactive polysaccharide, and two lipide, constituents. *Br. melitensis* was distinguished further from the other two species by the occurrence of a non-protein, non-polysaccharide, precipitating antigen of a type hitherto undescribed.

Preparation of Alcoholic Precipitate of *Brucella abortus*

Przesmycki's method was closely followed at first and a precipitate was produced from *Br. abortus* with which experimental work was carried on. It was found that certain steps could be omitted or combined without making any difference in the final product. The method finally adopted was as follows: *Br. abortus* was grown for three to four days on liver agar, pH 6.6, in flat bottles. It was washed off with 25 cc. of sterile water to each bottle. The bottles had a surface area of about 25 sq. in. The suspension was then shaken for 24 hr. with glass beads in a revolving shaker. It was then heated in flowing steam for five minutes after the temperature of the fluid reached its maximum. As ordinary methods of centrifugation are unsatisfactory with *Brucella*, the suspension was passed through a Sharples supercentrifuge running at 40,000–50,000 r.p.m. This usually gave a clear fluid or one with only slight cloudiness. This was then filtered through a fine Mandler candle and a crystal-clear product was obtained. Five volumes of 95% ethyl alcohol was added to this filtrate, which was then allowed to stand at room temperature until the next day. A fluffy precipitate settled out overnight. This was collected by centrifugation, washed in a mixture of equal parts of alcohol and ether, again collected by centrifugation, and visible fluid evaporated off in the incubator. This usually took two to three hours, after which the drying was completed *in vacuo* over phosphorus pentoxide. The resultant material was ground in an agate mortar and stored in rubber-stoppered vials. For use in the following experiments the powder was suspended in distilled water by grinding. It did not all go into solution, but as a filtrate was as active as the unfiltered suspension, as will be shown later, it was evident that the active portion was readily soluble. There were always particles in an unfiltered suspension, no matter how finely it had been ground. Acetic acid and sodium bicarbonate were both tried in attempts to dissolve these particles but some always remained. Twenty-five flasks of liver agar (625 sq. in. of surface) yielded about one gram of alcoholic precipitate.

Intraperitoneal Injection of Alcoholic Precipitate in Guinea Pigs

In order to test the toxicity of the various lots of alcoholic precipitate, guinea pigs were given varying doses by intraperitoneal injection. Temperatures were taken and the general condition of the animals observed. As will be seen from the following data some guinea pigs died and others recovered. In the immunity experiments, where several injections were given, there was usually a loss of weight and condition, even though the dosage was small, and some died after a number of sub-lethal injections. At first the required dose was injected in 5 or even 10 cc. of fluid, but latterly in 2.0 cc. It was not filtered before injection, except where this procedure is specified, and, as will be seen, filtration did not affect the final suspension. While the active part was undoubtedly in solution there was a certain amount of undissolved material in fine particles. The precipitate seemed to be more readily suspended in water than in salt solution.

Experiment 1. Intraperitoneal injection of 0.005 gm. of Lot 1. A 350 gm. guinea pig was given an intraperitoneal injection of 0.005 gm. of Lot 1 in 1.0 cc. water. Pre-injection temperature was 104.0° F. One hour later the temperature was 102.4, two hours after injection it was the same, and the following morning it was 103.0. Not much disturbance was shown from this small dose, but an injection of 0.02 gm. proved fatal.

Experiment 2. Intraperitoneal injection of 0.01 gm. of Lot 2. A 400 gm. guinea pig was injected by the intraperitoneal route with 0.01 gm. of Lot 2 in 10 cc. saline. The initial temperature was 104.0° F. Seven hours later the temperature was 98.4 and the guinea pig was greatly distressed. Heart action was slowed and thudding. Breathing quickened. The following morning this animal appeared normal and the temperature was 103.0.

The same precipitate was suspended in saline. Two lots of 0.04 gm. in 10 cc. were left in an ice chest overnight. The following day they were diluted to 20 cc. with saline. One lot was then filtered several times through a micro-filter packed with asbestos. This did not clear the solution which was then passed through a Mandler candle, and a crystal-clear solution the color of pale broth was obtained. Ten cc. of this filtrate and 10.0 cc. of the unfiltered suspension (each containing 0.02 gm.) were injected into the abdominal cavity of guinea pigs. Both animals weighed about 350 gm. Table I shows the temperature of these animals. The first temperature was taken at time of injection.

TABLE I

TEMPERATURES OF GUINEA PIGS INJECTED WITH LOT 2, FILTERED AND UNFILTERED

—	2:00 p.m.	3:00 p.m.	9:15 p.m.	10:00 a.m.
Filtered	101.0	103.0	100.0	103.8
Unfiltered	102.0	104.6	99.6	103.0

It will be seen that there was no practical difference between the filtered and unfiltered product. General symptoms were the same in both animals; tumultuous, thumping heart action and accelerated breathing. Both recovered.

Experiment 3. Intraperitoneal injection of filtered and unfiltered Lot 4 alcoholic precipitate. Lot 3 of the alcoholic precipitate was lost through breaking of the desiccator while under vacuum. Lot 4 was injected into two guinea pigs, one portion filtered as in the previous experiment and the other portion unfiltered. The dose was 0.02 gm. in 10 cc. of water in each case. Table II shows the temperature changes in these animals.

TABLE II

TEMPERATURE CHANGES PRODUCED BY LOT 4 FILTERED AND UNFILTERED

	Mar. 23			Mar. 24	Mar. 25
	11:30 a.m.	2:00 p.m.	5:30 p.m.	9:00 a.m.	9:00 a.m.
Filtered	102.2	102.0	96.0	96.6	102.6
Unfiltered	102.6	101.4	97.4	101.4	102.0

Both animals showed the same symptoms as in the previous experiment but had recovered the second day after the injection. Again there was no significant difference between the filtered and the unfiltered suspension.

Experiment 4. Effect of dialysis on alcoholic precipitate. One tenth of a gram of Lots 4 and 5 was suspended in 12.5 cc. saline. Six cc. of each suspension was placed in a collodion sac in running tap water. The balance of each sample was held in a test tube in the water so as to be kept under the same conditions. The following day each portion was brought to 10.0 cc. containing 0.05 gm. and four guinea pigs received intraperitoneal injections of 7.0 cc. each (0.035 gm. alcoholic precipitate). Table III gives the temperature reactions of these animals.

TABLE III

TEMPERATURE CHANGES PRODUCED BY DIALYZED AND UNTREATED SUSPENSIONS OF LOTS 4 AND 5

	April 12				April 13				April 14	
	11:00 a.m.	1:00 p.m.	4:00 p.m.	5:30 p.m.	9:00 a.m.	12:00 m.	3:15 p.m.	5:00 p.m.	9:00 a.m.	12:00 m.
LOT 4										
Untreated	102.0	98.0	94.0	94.0	94.0	94.0	dead			
Dialyzed	101.8	99.4	96.0	96.0	102.2	103.0	103.0	103.0	101.4	101.6
LOT 5										
Untreated	102.0	98.0	94.0	94.0	94.0	94.0	94.0	94.0	dead	
Dialyzed	101.6	99.0	97.4	97.0	97.6	98.4	101.4	102.8	102.6	102.6

In this experiment, while all the animals were ill, the two that received the untreated suspension died and the two treated with dialyzed material recovered. The dialyzed suspensions had increased in volume and both sacs were shown to be readily porous for sodium chloride when tested afterwards. The color was not removed from the dialyzed fluids. Dialysis did remove some of the temperature-reducing and lethal qualities of these precipitates. This is clearly shown in Fig. 1.

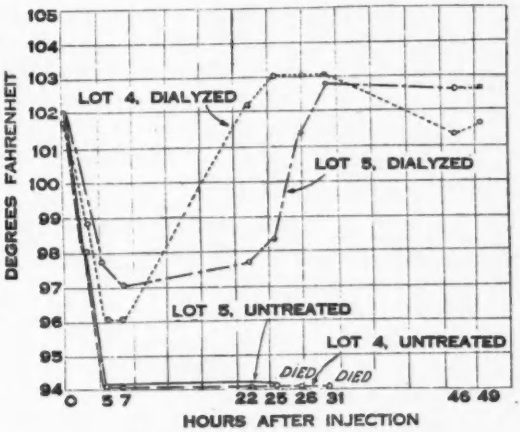

FIG. 1. *Intraperitoneal injections of dialyzed and untreated precipitate. Temperatures of guinea pigs.*

Experiment 5. Intraperitoneal injection of 0.04 and 0.01 gm. of Lot 6 alcoholic precipitate. Two 350 gm. guinea pigs were given intraperitoneal injections of 0.04 and 0.01 gm. of Lot 6 alcoholic precipitate in 5 cc. water. Table IV gives the temperatures of these animals.

TABLE IV

TEMPERATURE OF GUINEA PIGS INJECTED WITH LOT 6 ALCOHOLIC PRECIPITATE

	10:00 a.m.*	11:15 a.m.	1:30 p.m.	3:00 p.m.	4:30 p.m.	9:00 a.m.	12:00 m.	3:30 p.m.	5:00 p.m.	9:00 a.m.
0.04 gm.	103.0	99.4	94.8	94.8	94.0	dead				
0.01 gm.	102.4	102.2	95.0	96.1	97.0	96.2	94.0	94.0	94.0	dead

**Pre-injection temperatures.*

The usual symptoms were manifested by these animals. That receiving the larger dose died during the night while the one receiving the smaller dose lived for another day.

Experiment 6. Intraperitoneal injection of Lot 7 alcoholic precipitate. Two guinea pigs weighing about 360 gm. received the same injection as in the previous experiment, namely, 0.04 and 0.01 gm. of Lot 7 alcoholic precipitate in 2 cc. salt solution. Temperatures are shown in Table V.

TABLE V

TEMPERATURES OF GUINEA PIGS INJECTED WITH LOT 7 ALCOHOLIC PRECIPITATE

	May 18						May 19		May 20
—	10:00 a.m.	11:00 a.m.	12:00 m.	2:30 p.m.	3:45 p.m.	4:45 p.m.	9:30 a.m.	11:30 a.m.	10:00 a.m.
0.04 gm.	103.8	102.0	100.4	100.4	100.4	100.0	dead		
0.01 gm.	103.0	103.0	104.8	100.4	98.0	96.6	95.8	94.0	dead

The larger dose did not produce the usual rapid temperature drop but this animal died a day sooner than that receiving the smaller dose.

Experiment 7. Dialysis of Lot 7 alcoholic precipitate. Two lots of 0.05 gm. in 10 cc. water were prepared. One portion was dialyzed in running tap water for 48 hr. The other was immersed in a test tube in the same water. Two guinea pigs were given intraperitoneal injections of 9.0 cc. containing 0.03 gm. They were injected at noon, both temperatures dropped equally rapidly and both animals were dead the following morning. The dialyzing sac was not prepared according to any standard but it was shown to be readily permeable to sodium chloride.

Experiment 8. Intraperitoneal injections of alcoholic precipitate prepared with varying volumes of alcohol. Lot 8 was divided into three portions prior to the precipitating stage and to these were added 2.5, 5 and 10 volumes of 95% ethyl alcohol. The relative weights obtained from 340 cc. of filtrate by each method were: From 2.5 volumes, 0.360 gm.; from five volumes, 0.425 gm.; and from 10 volumes, 0.540 gm. Three guinea pigs were injected with 0.62 gm. of each precipitate in 2 cc. water at 2:00 p.m. All showed the same rapid drop in temperature before night, and all were dead the following morning. Variation from 2.5 to 10 volumes of alcohol made no apparent difference in the temperature-reducing and lethal qualities of the end product. Increase in alcohol did increase the yield of precipitate.

Experiment 9. Intraperitoneal injection of 0.02 gm. of Lot 9 alcoholic precipitate. Two guinea pigs were injected with this lot of alcoholic precipitate. Temperatures are shown in Table VI.

TABLE VI

TEMPERATURES OF GUINEA PIGS INOCULATED WITH LOT 9

—	9:15 a.m.	11:15 a.m.	1:45 p.m.	3:45 p.m.	5:00 p.m.	12:00 m.	9:00 a.m.	11:45 a.m.	2:00 p.m.
1	102.8	100.0	95.0	94.0	dead				
2	103.8	98.2	96.0	97.4	96.0	98.0	97.8	99.2	101.0

One guinea pig died the day of injection. The other was very ill but eventually recovered.

Symptoms and post-mortem findings in guinea pigs. Intraperitoneal injections of very small doses of alcoholic precipitate produced no other effects than a slight elevation of temperature. Larger doses caused nervous symptoms followed by a rapid fall in temperature, loss of power in the limbs, distention of the abdomen and death in from six hours to several days. In those animals that died quickly the only change was a severe inflammation of the peritoneum. In those that lived for a day or more a fibrinous exudate was observed on the liver and a quantity of pus was present. In most cases this was sterile, but from some, colon bacilli, staphylococci and other bacteria were isolated.

Effect of alcoholic precipitate on hypersensitive human subject. It is interesting to note that preparation of this material gave rise to symptoms simulating undulant fever in a hypersensitive person. The subject had a history of what was probably a mild attack of undulant fever some years earlier and had given a positive agglutination and complement fixation reaction in 1 : 100 for the last four years. Tests before this time were not made. The blood showed marked phagocytic activity by Huddleson's method (1). Exposure appeared to desensitize for several weeks. Symptoms were, chill, slight increase of temperature (2°), intense lassitude, double vision in some attacks, pain and stiffness in the small of the back and sometimes in the knee joints, intense sweating during the night, quickened breathing and a thudding heart action. The day after exposure the symptoms abated, leaving a feeling of marked lassitude. These symptoms developed on three occasions, following the grinding or evaporation of products of *Br. abortus* in quantity. The subject is not affected by the ordinary laboratory handling of the organism.

Biochemical Nature of Alcoholic Precipitate

A suspension of 0.02 gm. of Lot 2 in 20 cc. salt solution (1 : 100) was allowed to stand in the refrigerator overnight. It was filtered through a fine Mandler candle. Dr. C. S. Hanes of the Foundation examined this solution and reported as follows:—

Hopkins Cole Test—Faint positive. *Millon's Test*—Negative. *Biuret*—Faint violet.

Dr. Hanes tried to precipitate the remaining fluid with 60% saturated ammonium sulphate solution. It became slightly cloudy but did not advance any further and no precipitate was formed.

A suspension of 0.2 gm. in 50 cc. saline was prepared and filtered through a Mandler candle. Five-cc. lots of the filtrate were treated by Dr. Hanes with ammonium sulphate, trichloracetic acid, trypsin, heat and hydrochloric acid, and heat and sodium hydroxide. The ammonium sulphate produced a precipitate. Trichloracetic acid produced a faint opalescence with 5% and 12% after four hours. Hydrochloric acid and heat did not cause precipitation (5 cc. sol. + 2 cc. *N* hydrochloric acid). Sodium hydroxide and heat (5 cc. sol. + 2 cc. *N* sodium hydroxide) caused a heavy flocculent precipitate. This disappeared on neutralization with hydrochloric acid. Injection experiments, skin tests and complement fixation tests were carried out. The suspension, unfortunately, appeared to have been too weak for its purpose and rather indefinite results were obtained.

Lot 12 precipitate was divided and 0.45 gm. was suspended in 200 cc. distilled water. After about an hour, with intermittent shaking, it was filtered through paper and a fine Mandler candle. Five volumes of 95% alcohol was added to the filtrate, which was allowed to stand overnight. The fluid became cloudy but no precipitate was formed. *N* sodium hydroxide was added and a precipitate began to form in a few minutes. This was allowed to settle out, collected by centrifugation and dried, first in the incu-

bator and then over phosphorus pentoxide *in vacuo*. The supernatant fluid was filtered through a fine candle to remove any particles of precipitate. The first precipitate, the second precipitate, and the residue of the supernatant fluid were turned over to Dr. A. D. Barbour for examination. Guinea pigs were injected with 0.01 gm. of each product in 2.0 cc. water. The original precipitate caused a fall in temperature in the injected guinea pig from 103.0 to 99.8° F. The reprecipitated material, contrary to expectations, was not toxic. There was but slight change in temperature, less than a degree, during the period of observation. The residue of the supernatant fluid caused a slight rise of temperature. This material of course contained the added sodium hydroxide, and other soluble material, so that the actual amount of active material would be less than 0.01 gm. Dr. Barbour's report on the three samples is given in Table VII.

TABLE VII

Test	Given by	1 Original precipitate	2 Reprecipitated	3 Residue
Biuret	All proteins	?	—	?
Xanthoproteic	Thyosine, Phenylalamine, Tryptophane	?	—	+ (weak)
Millon	Tyrosine	—	—	—
Ferrocyanide	All proteins	?	—	?
Molisch	All carbohydrates	++++	++++	++++

Dr. Barbour states: "I would conclude from this that your material consists almost entirely of carbohydrates, with possibly a trace of protein in samples 1 and 3."

A mixture of several lots of alcoholic precipitate was next prepared and 0.45 gm. was ground up in water and the volume brought to 200 cc. This was allowed to stand overnight, filtered, and 10 cc. *N* sodium hydroxide and 1000 cc. alcohol were added. The precipitate that formed was collected and dried, as was also the insoluble material on the filter paper. Guinea pigs were given intraperitoneal injections of these substances and the original precipitate as follows:—

No. 1. 0.015 gm. of insoluble material.

No. 2. 0.02 gm. of reprecipitated material.

No. 3. 0.02 gm. of original precipitate.

The temperature reactions of these animals are shown in Table VIII.

TABLE VIII

TEMPERATURES OF GUINEA PIGS INJECTED WITH PRECIPITATES AND INSOLUBLE MATERIAL

	10:00 a.m.	12:00 m.	2:00 p.m.	4:00 p.m.	5:00 p.m.	9:00 a.m.	12:00 m.	2:00 p.m.	4:00 p.m.	9:00 a.m.	12:00 m.
1	101.6	94.8	98.6	93.0	93.0	93.0	94.0	94.0	94.0	98.4	100.6
2	102.0	93.0	93.0	93.0	93.0	dead					
3	101.8	98.4	100.4	95.0	94.4	96.6	98.6	100.4	100.4	101.4	102.0

The temperatures of all three guinea pigs dropped and all were ill, but those receiving the insoluble material and the original precipitate recovered, while the animal that was injected with the reprecipitated material died.

Summary

An alcoholic precipitate was prepared from a filtrate of *Brucella abortus* suspension. Intraperitoneal injection of this material in guinea pigs produced peritonitis and death where the dosage was sufficiently large.

Seventeen guinea pigs received intraperitoneal injections of untreated alcoholic precipitate ranging from 0.005 to 0.04 gm. Twelve died and five lived. The animal receiving the smallest dose did not show a drop below the normal range of temperature. The others all showed a drop in temperature. Three animals received 0.01 gm. Two of these died. Eight were given 0.02 gm. and in this group five died. The five guinea pigs that received 0.03 to 0.04 gm. all died.

Dialysis reduced the temperature-lowering and lethal fraction in the first experiment with injections of 0.035 gm. but in the second experiment, although the material was dialyzed longer, both animals died from an injection of 0.03 gm. The dialyzing sacs were not standardized in either case.

Filtration of the suspension of alcoholic precipitate through a Mandler candle before injection did not modify the effect on injected guinea pigs, indicating that the active part of the material readily went into solution in water.

The preparation of the precipitate gave rise to symptoms simulating undulant fever in a hypersensitive human subject. The attacks appeared to desensitize the subject for several weeks.

Preliminary biochemical examination suggested that the precipitate consisted almost entirely of carbohydrates. The original precipitate and the residue of the supernatant fluid showed a trace of protein that was absent in reprecipitated material.

Acknowledgments

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A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

II. COMPLEMENT FIXATION AND INTRADERMAL TESTS WITH ALCOHOLIC PRECIPITATE[1]

By Ronald Gwatkin[2]

Abstract

Alcoholic precipitates of *Brucella abortus* showed high antigenic qualities in the complement fixation test. Dialysis and filtration did not lower this activity. The precipitates gave rise to reactions in infected guinea pigs, in dilutions which produced no change in normal animals. Filtration did not modify the results. In two out of three samples dialysis did not lower the activity of the suspensions as skin test antigens. The addition of formol did not modify the skin reactions.

Introduction

In a previous paper (1) the writer described the preparation, biochemical nature and toxicity of an alcoholic precipitate prepared from a filtered suspension of *Br. abortus*. This article deals with the precipitate as an antigen in the complement fixation test, and also as an agent for the intradermal test in normal and infected guinea pigs.

Complement Fixation Tests with Alcoholic Precipitate

Complement fixation tests were carried out with the various lots of alcoholic precipitates, untreated, dialyzed and filtered. Details of these are given in the following experiments. In general the suspensions made fairly good antigens, and could be used sufficiently dilute to avoid anticomplementary reactions. At first various strengths were used but latterly 0.01 gm. was suspended in 2 cc. saline (1:200), and from this, dilutions of 1:25, 1:100 and 1:500 were made, which gave final dilutions of 1:5000, 1:20,000 and 1:100,000 of the precipitate. In order to use small quantities of precipitate, 0.01 gm. was suspended in 2 cc. saline. To this was added 48 cc. saline making the 1:25 dilution. Part of this was filtered and then diluted to make the 1:100 and 1:500 dilutions of the suspension. The unfiltered suspension was diluted at the same time. One cc. of each dilution was used in the test, which was the one regularly used in this laboratory for routine diagnosis and experimental work. All alcoholic precipitate antigens were heated just before use for 30 min. at 56° C.

Experiment 1. Complement fixation test on Lot 1. A suspension of 0.1 in 5 cc. was prepared. This was diluted 1:25 and 1:1000 (1:1250 and 1:50,000 of the precipitate). Results are shown in Table I.

[1] *Manuscript received September 27, 1934.*
Contribution from Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Canada.

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TABLE I

COMPLEMENT FIXATION TEST OF LOT 1 ALCOHOLIC PRECIPITATE

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500	1 : 5000
Alcoholic precipitate 1 : 25	4	4	4	4	4	0	0	0
1 : 1000	0	0	0	0	0	0	0	0
Regular antigen 1 : 25	4	4	4	4	4	4	3	0

In the tables showing results of complement fixation tests: 4 = complete fixation, 3 = 75% fixation, 2 = 50% fixation, 1 = 25% fixation, 0 = no fixation.

The spread between the two dilutions was too great and a 1:50,000 dilution of the precipitate did not produce fixation with the serum employed, which had a titre of 1:1000 by the regular test.

Experiment 2. Complement fixation test on Lots 1 and 2, filtered and unfiltered. A suspension of 0.01 gm. in 2 cc. (1:200) was diluted to 1:25 and 1:100 (dilutions of 1:5000 and 1:20,000 of the precipitate). One portion was filtered through a Mandler candle and the other was left unfiltered. The results of this test are shown in Table II.

TABLE II

COMPLEMENT FIXATION TEST ON LOTS 1 AND 2, FILTERED AND UNFILTERED

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Al. ppt. 1 1 : 25	4	4	4	4	0	0	0
1 : 100	4	4	4	4	4	0	0
Al. ppt. 1 (filtered)							
1 : 25	4	4	4	4	0	0	0
1 : 100	4	4	4	4	4	0	0
Al. ppt. 2 1 : 25	4	4	4	4	4	0	0
1 : 100	4	4	4	4	4	4	0
Al. ppt. 2 (filtered)							
1 : 25	4	4	4	4	4	0	0
1 : 100	4	4	4	4	4	4	0
Regular antigen							
1 : 15	4	4	4	4	4	4	0

It will be seen from Table II that the higher dilution of each precipitate gave one tube higher fixation than the lower dilution. Presumably this is due to some inhibitory substance that disappears in the higher dilutions.

Experiment 3. Complement fixation test on Lot 4, filtered and unfiltered. A suspension of 0.01 gm. in 2 cc. was made and the same dilutions prepared as in the previous experiment. The results are shown in Table III.

TABLE III

COMPLEMENT FIXATION TEST ON FILTERED AND UNFILTERED SAMPLES OF LOT 4

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Filtered	1 : 25	Anticomplementary						
	1 : 100	4	4	4	4	2	0	0
Unfiltered	1 : 25	Anticomplementary						
	1 : 100	4	4	4	2	0	0	0
Regular antigen	1 : 15	4	4	4	4	4	4	0

It will be seen in Table III that, although the difference was slight, the filtered suspension showed more fixation than the unfiltered. Neither gave as high a reaction in comparison with the regular bacterial antigen as did Lot 2 in Table II. This precipitate was anticomplementary in 1:25 dilution of the suspension.

Experiment 4. Complement fixation test on Lots 4 and 5, dialyzed and untreated. One-tenth gm. of each precipitate was suspended in 12.0 cc. saline. Six cc. of each was placed in a collodion sac and dialyzed overnight in running water. The remainder was immersed in a test tube in the water to have the holding conditions the same. Both lots were brought next day to 10 cc., each containing 0.05 gm. Dilutions of 1:25, 1:100 and 1:500 were made from each suspension, representing dilutions of 1:5000, 1:20,000 and 1:100,000 of the precipitate. The results are given in Table IV.

TABLE IV

COMPLEMENT FIXATION TEST ON DIALYZED AND UNTREATED SAMPLES OF LOTS 4 AND 5

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
No. 4 Untreated							
1 : 25	Anticomplementary						
1 : 100	4	4	4	1	1	0	0
1 : 500	0	0	0	0	0	0	0
No. 4 Dialyzed							
1 : 25	Anticomplementary						
1 : 100	4	4	4	1	0	0	0
1 : 500	0	0	0	0	0	0	0
No. 5 Untreated							
1 : 25	4	4	4	4	4	0	0
1 : 100	4	4	4	4	4	1	0
1 : 500	3	3	4	4	4	4	0
No. 5 Dialyzed							
1 : 25	4	4	4	4	4	1	0
1 : 100	4	4	4	4	4	2	0
1 : 500	2	3	4	4	4	4	0
Regular antigen							
1 : 15	4	4	4	4	4	4	0

Lot 4 was again anticomplementary in the lowest dilution. The highest dilution gave the best fixation with both lots. No. 5 was equal to the bacterial antigen. The dialyzed product was the same as the untreated one, any differences being too slight to be considered. Reference might be made here to another experiment (1), in which these same dialyzed and untreated precipitate suspensions were injected into guinea pigs by the intra-abdominal route. Both dialyzed-suspension animals lived while the untreated-suspension pair died. It will be noticed that Lot 5 shows a prezone phenomenon in the first two tubes.

Experiment 5. Complement fixation test on Lot 6. A suspension of 0.01 in 2 cc. was diluted 1:25, 1:100 and 1:500 to make precipitate dilutions of 1:5000, 1:20,000 and 1:100,000. Results are shown in Table V.

TABLE V

COMPLEMENT FIXATION TEST ON LOT 6

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
1 : 25	4	4	4	0	0	0	0
1 : 100	4	4	4	3	0	0	0
1 : 500	4	4	4	4	0	0	0
Regular antigen 1 : 15	4	4	4	4	4	4	0

As with Lots 4 and 5, the best fixation was shown in the highest dilution. This lot did not equal the bacterial antigen with the serum employed.

Experiment 6. Complement fixation test with dialyzed and untreated samples of Lot 7. A suspension of 0.01 gm. in 2.0 cc. was diluted 1:25, 1:100, and 1:500. The results of this test are given in Table VI.

TABLE VI

COMPLEMENT FIXATION TEST ON LOT 7

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
1 : 25	4	4	2	2	0	0	0
1 : 100	4	4	4	4	0	0	0
1 : 500	4	4	4	4	4	0	0
Regular antigen 1 : 15	4	4	4	4	4	4	0

The highest dilution again gave the best fixation but did not equal the bacterial suspension antigen.

Two lots of 0.05 gm. of this precipitate were suspended in 10.0 cc. water. One lot was dialyzed and the other left in a test tube immersed in the water. The fluid had increased in the sac to 15.0 cc. at 42 hr., so the suspension in the tube was also brought to this quantity, making both dilutions 1:300. Further dilutions were made from this of 1:15, 1:60 and 1:300 (final dilutions of the precipitate of 1:4500, 1:18,000 and 1:90,000). Results of this test are shown in Table VII.

The lowest dilution (1:4,500) was anticomplementary. A dilution of 1:5000 of this precipitate a week previously had not been anticomplement-

TABLE VII

COMPLEMENT FIXATION TEST ON DIALYZED AND UNTREATED SAMPLES OF LOT 7

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000
Dialyzed	1 : 15	Anticomplementary					
	1 : 60	4	4	4	0	0	0
	1 : 300	4	4	4	4	0	0
Untreated	1 : 15	Anticomplementary					
	1 : 60	4	4	4	0	0	0
	1 : 300	4	4	4	3	0	0
Regular antigen	1 : 15	4	4	4	4	4	0

ary. It is possible that this tendency might have developed while the material was in suspension for two days. The previous lot was suspended just before it was required. Both had been heated just before use for 30 min. at 56° C. There was no real difference between the dialyzed and untreated material, and what slight difference there was favored the dialyzed material.

Experiment 8. Complement fixation test on Lots 8, 9, 10 and 11. Lot 8 was divided into three portions and precipitated with 2.5, 5 and 10 volumes of alcohol. Lot 9 was made in the ordinary way with five volumes of alcohol as were also Lots 10 and 11. Suspensions of 0.01 gm. of precipitate were treated in the ordinary way and results are shown in Table VIII.

TABLE VIII

COMPLEMENT FIXATION TEST ON LOTS 8, 9, 10 AND 11

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500	1 : 5000
Lot 8 (2.5 vols.)	1 : 25	Anticomplementary							
	1 : 100	4	4	4	3	0	0	0	0
	1 : 500	4	4	4	4	0	0	0	0
Lot 8 (5 vols.)	1 : 25	Anticomplementary							
	1 : 100	4	4	4	0	0	0	0	0
	1 : 500	4	4	4	4	0	0	0	0
Lot 8 (10 vols.)	1 : 25	Anticomplementary							
	1 : 100	4	4	4	0	0	0	0	0
	1 : 500	4	4	4	4	0	0	0	0
Lot 9	1 : 25	4	4	4	3	0	0	0	0
	1 : 100	4	4	4	4	0	0	0	0
	1 : 500	4	4	4	4	4	1	0	0
Regular antigen	1 : 15	4	4	4	4	4	4	0	0
Lot 10	1 : 25	4	4	4	4	4	0	0	0
	1 : 100	4	4	4	4	4	0	0	0
	1 : 500	4	4	4	4	4	4	0	0
Lot 11	1 : 25	4	4	4	4	2	0	0	0
	1 : 100	4	4	4	4	4	2	0	0
	1 : 500	4	4	4	4	4	3	0	0
Regular antigen	1 : 15	4	4	4	4	4	4	4	0

There was very little difference between the precipitates produced by 2.5, 5 and 10 volumes of alcohol. The highest dilution in each case gave identical results and the lowest was anticomplementary. In all lots the best fixation was shown by the highest dilution of the precipitate.

Intradermal Tests with Alcoholic Precipitate

Infected and normal guinea pigs and rabbits were tested with suspensions of alcoholic precipitate to determine the skin response in these animals. It is realized that skin tests in small animals may not be applicable to cows, but they were carried out for the purpose of obtaining as much information as possible about the antigenic qualities of these precipitates.

Experiment 9. Intradermal test of infected and normal guinea pigs with Lot 1 alcoholic precipitate. Two infected and two normal guinea pigs were shaved over the abdominal region. Each guinea pig was injected with 0.1 cc. of a suspension of 0.005 gm. in 1.0 cc. saline (1:200). The following day the infected guinea pigs showed a small but distinct inflammatory reaction. The normal animals showed no reaction. The swelling on the positive animals persisted for several days.

Experiment 10. Intradermal test of normal and infected guinea pigs with filtered and unfiltered suspensions of Lot 2 alcoholic precipitate. A suspension of 0.01 gm. in 10.0 cc. saline (1:1000) was prepared and intradermal injections were made in the shaved abdominal region. At 19 hr. there was a slight reaction in the infected animal. The reaction was quite distinct at 24 hr. and persisted for several days. There was no reaction in the normal guinea pig.

A suspension of 0.01 gm. in 2 cc. (1:200) produced a well-marked swelling in another infected guinea pig but also caused a reaction in a normal animal.

A suspension of 0.02 gm. in 10.0 cc. saline (1:500) was prepared. Half of it was filtered through a fine Mandler candle. A normal and an infected guinea pig were injected intradermally in the abdominal region with 0.1 cc. of filtered and unfiltered suspension. Both produced a reaction in the infected animal that was observable at 7 hr. and well marked at 24, 48 and 72 hr. Neither product produced any reaction in the normal animal.

Experiment 11. Intradermal test with Lot 4, filtered and unfiltered. A suspension of 0.02 gm. in 10.0 cc. saline (1:500) was divided, and one portion was filtered through a fine Mandler candle. Each was injected in the shaven skin of the abdomen of a normal and an infected guinea pig. In six hours there was a reaction to both filtered and unfiltered suspensions in the infected guinea pig but not in the normal animal. This condition was the same at 24 and 48 hr.

Experiment 12. Intradermal tests of rabbits with Lots 4 and 5, dialyzed and untreated. One-tenth of a gram of each lot was suspended in 12.0 cc. salt solution. Six cc. of each suspension was placed in a collodion sac in running

tap water. The other 6 cc. was held in a test tube in the same water, in order to have keeping conditions as close as possible. The following day both lots were diluted to 10.0 cc. with saline (1:500). A normal and an infected rabbit were clipped and shaved over the abdominal area. One-tenth cc. of the dialyzed and untreated suspensions was injected into each rabbit. At 24 hr. all the suspensions showed a clear reaction, but untreated Lot 4 was very prominent. Pus appeared in this lesion at 48 hr. while the reaction from the dialyzed No. 4 sample had practically disappeared. At this time both dialyzed and untreated suspensions of Lot 5 showed a distinct reaction, no difference being observed between the two. *E. coli* was recovered from the untreated No. 4 lesion. The marked reaction was obviously due to this cause. Dialysis made no difference to Lot No. 5. The reaction from the dialyzed No. 4 sample had practically disappeared at 48 hr. but this could not be compared to the undialyzed portion on account of infection in that reaction site.

Experiment 13. Intradermal tests of guinea pigs with Lot 7, dialyzed and untreated. A dilution of 1:300 of dialyzed and untreated alcoholic precipitate was injected in an infected and a normal guinea pig. One-tenth cc. of each was injected in the shaven skin of the abdomen. The suspension had been dialyzed in running tap water for 48 hr. At 24 hr. there was some reaction from both suspensions in both the normal and infected guinea pigs. The swelling disappeared in the former but there were small, hard lumps from both dialyzed and untreated material on the infected pig four days after injection. Dialysis did not make any appreciable difference to the reaction of this material.

Experiment 14. Formolized and untreated suspensions of Lot 7. A suspension was made of 0.05 gm. of Lot 7 in 10.0 cc. water (1:500), which was divided into two lots. To one was added 0.25% of formol C.P. Both were held in the refrigerator for two days. One-tenth cc. of each was injected into the shaven skin of a normal and an infected guinea pig. There was no difference between the reaction of the formolized and untreated filtrate in the infected guinea pig and neither produced reactions in the negative animal.

Summary

Alcoholic precipitate of *Br. abortus* showed high antigenic qualities in the complement fixation test. Dilutions of 1:100,000 as a rule gave the best fixation, although there were some exceptions. Some of the precipitates were anticomplementary in a dilution of 1:5000. Dialysis did not lower the antigenic quality of a suspension of precipitate. The collodion sacs were not standardized but were readily permeable to sodium chloride. Filtering through a fine Mandler candle did not lower the antigenic quality of a suspension of precipitate. Precipitation of the filtrate with 2.5, 5 and 10 volumes of alcohol did not modify the precipitate as an antigen in the complement fixation test.

The intradermal injection of alcoholic precipitate gave rise to an inflammatory reaction in infected guinea pigs in dilutions of 1:500 and 1:1000.

These dilutions did not give rise to reactions in normal animals. One of two lots of precipitate diluted 1:200 caused an inflammatory reaction in a normal guinea pig, as did also a 1:300 dilution of another lot.

Filtering through a fine Mandler candle did not affect the activity of a suspension as an antigen in the skin test.

Skin reactions were not influenced by dialysis of two out of three lots of precipitate. The third sample appeared to have been reduced in activity but no comparison could be made with the injection of untreated suspension as infection occurred at this site of injection.

The addition of 0.25% formalin did not influence the activity of a sample of precipitate as an antigen in the skin test.

Acknowledgments

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Reference

1. GWATKIN, RONALD. A study of various fractions of *Brucella abortus*. I. Preparation, toxicity and biochemical nature of alcoholic precipitate. Can. J. Research, 12: 115-124. 1935.

A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

III. IMMUNITY EXPERIMENTS WITH ALCOHOLIC PRECIPITATE[1]

By Ronald Gwatkin[2]

Abstract

Brucella antiserum protected guinea pigs against intraperitoneal injections of alcoholic precipitate. After absorption with *Brucella abortus* this serum was not protective. Normal serum also failed to modify the toxicity of the precipitate. Alcoholic precipitate was as efficient as whole culture in removing antibodies *in vitro*, as judged by serological tests. Intraperitoneal injections of precipitate in small animals stimulated agglutinin production and opsonocytophagic activity, but did not protect guinea pigs against infection by eye with *Brucella abortus* or prevent abortions in infected animals. Oral administration of precipitate did not give rise to symptoms in guinea pigs.

Introduction

In previous papers (2, 3) the writer has described the preparation of an alcoholic precipitate from a filtered suspension of *Br. abortus*, the biochemical nature of the product, its use as an antigen in the complement fixation test and in skin tests on normal and infected guinea pigs and rabbits.

This article records the effect of anti-abortus serum in neutralizing the toxicity of the precipitate, oral administration of precipitate, and its use as an antigen to produce active immunity in guinea pigs.

Effect of Anti-abortus Serum on *Br. abortus* Alcoholic Precipitate

An alcoholic precipitate was obtained from a colon bacillus. This was even more toxic for guinea pigs than the *Brucella* precipitate. In view of this it was necessary to test the specificity of the *Brucella* product, which was done by protection tests of guinea pigs with anti-abortus serum from various animals.

Experiment 1. In this experiment an old horse serum was employed. It had been preserved with merthiolate 1 : 5000. It was filtered through a Mandler candle before use. It was found too late that this serum had developed a temperature-reducing quality and it was therefore unsuitable for the purpose, and did not protect the injected guinea pigs.

Experiment 2. Fresh citrated rabbit blood. Citrated blood from rabbits that had received several injections of *Br. abortus* was collected. Guinea pigs were given intraperitoneal injections as follows:—

No. 1. 5 cc. whole, citrated blood.

No. 2. 5 cc. whole, citrated blood + 0.04 gm. Lot 5 precipitate.

No. 3. 5 cc. water + 0.04 gm. Lot 5 precipitate.

[1] *Manuscript received September 27, 1934.*
Contribution from Dept. of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Canada.
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Table I shows the temperatures of these animals. The first, at noon, is the pre-injection temperature.

TABLE I

TEMPERATURES OF GUINEA PIGS IN EXPERIMENT 2

	April 17			April 18			April 19–20		
	12:00 m.	2:30 p.m.	5:15 p.m.	10:00 a.m.	12:30 p.m.	5:00 p.m.	10:00 a.m.	4:45 p.m.	9:00 a.m.
No. 1	102.6	95.0	97.0	102.8	103.0	102.2	102.0	102.0	102.0
No. 2	102.0	94.0	94.0	102.0	103.4	104.4	102.8	102.4	102.2
No. 3	103.0	94.0	96.4	94.0	94.0	94.0	100.0	101.0	102.0

Rabbit blood alone caused some drop in temperature. The temperature of the blood-and-precipitate animal dropped to 94° but rose to normal the next day. Most of the animals that recover from injections of precipitate show an increase above normal temperature following the subnormal effect. The precipitate-alone animal remained low for a day longer than the others, but all three recovered.

Experiment 3. Effect of intraperitoneal injections of water, sodium citrate and broth in guinea pigs. It was considered necessary at this time to try the effect of water, sodium citrate solution and broth by intraperitoneal injection. No. 1 was given 5 cc. tap water. No. 2 was given 5 cc. tap water containing 0.1 gm. sodium citrate and No. 3 was injected with 5 cc. beef broth, pH 7.8. The temperatures of these animals are given in Table II.

TABLE II

TEMPERATURES OF GUINEA PIGS INJECTED WITH WATER, SODIUM CITRATE AND BROTH

	10:00 a.m.	12:30 p.m.	3:30 p.m.	5:00 p.m.	10:00 a.m.
No. 1	102.0	102.0	102.2	102.8	102.6
No. 2	101.0	102.0	101.4	101.8	101.6
No. 3	103.0	103.2	103.0	102.4	103.0

These guinea pigs showed no bad effects from the injections and the temperatures remained within normal range.

Experiment 4. Effect of heated rabbit serum on alcoholic precipitate. Serum from the rabbits mentioned in Experiment 2 was heated at 56° C. for 30 min. As in the former experiment, three guinea pigs were injected as follows:—

No. 1, weight 325 gm. 5 cc. heated serum alone.

No. 2, weight 312 gm. 5 cc. heated serum + 0.04 gm. Lot 5.

No. 3, weight 330 gm. 5 cc. water + 0.04 gm. Lot 5.

The temperatures of these animals are given in Table III and also the difference is very clearly shown in Fig. 1.

TABLE III

TEMPERATURES OF GUINEA PIGS INJECTED WITH SERUM AND ALCOHOLIC PRECIPITATE

	1:30 p.m.	4:45 p.m.	9:30 a.m.	12:00 m.	5:00 p.m.	9:30 a.m.
No. 1	102.0	101.6	101.8	102.2	101.6	101.8
No. 2	101.6	97.0	100.6	100.4	103.2	103.0
No. 3	102.0	95.0	94.0	dead		

The temperature of the serum-alone guinea pig did not go out of the normal range. The temperature of the serum-and-precipitate animal dropped to 97° but rapidly returned to normal. The precipitate-alone guinea pig died. Post-mortem examination of No. 3 showed peritonitis and fibrinous exudate over liver. Cultures were negative. Results were clear-cut in this experiment because there were no harmful results from the serum, which had been heated to remove its hemolytic activity.

Experiment 5. Hemolytic action of rabbit serum on guinea pig erethrocytes. Serums from the rabbits employed in Experiments 2 and 4 were tested for hemolytic activity on guinea-pig cells. In our earlier work (1, 4) it was found that fresh bovine serum was hemolytic and could not be safely injected into guinea pigs. The same procedure was employed. One-half cc. of each rabbit serum was added to 1.0 cc. of a 1% suspension of washed guinea-pig red cells and placed in a 37° C. water bath. The same quantity of these serums heated for 30 min. at 56° C. was added to 1.0 cc. quantities of cell suspension, and a tube of cell suspension only was included. The serum of the three rabbits which had been used for the protection test produced complete hemolysis of the guinea-pig cells in two minutes. Serum from another rabbit produced slight hemolysis in four minutes, but it was never complete. The bad effects from the use of the fresh, unheated rabbit serum were evidently due to hemolytic activity.

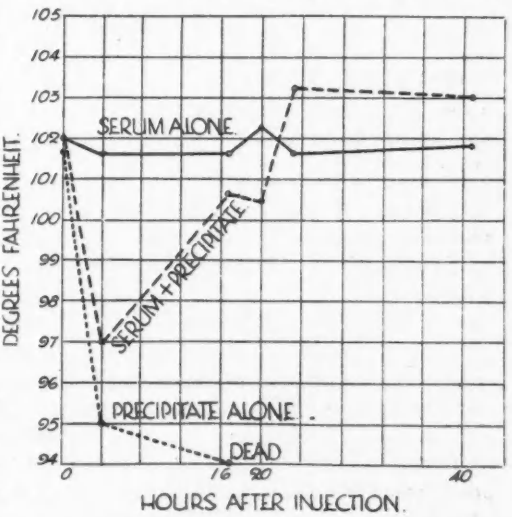

Fig. 1. Effect of heated rabbit serum on alcoholic precipitate. Temperatures of guinea pigs.

Experiment 6. Effect of heated normal and immune bovine serum on alcoholic precipitate. A mixture of Lots 3, 4, and 5, alcoholic precipitate was used.

The following suspensions were made and placed in the refrigerator overnight.

1. 0.04 gm. al. ppt. + 1.0 cc. pos. serum + 4 cc. water.
2. 0.04 gm. al. ppt. + 5.0 cc. pos. serum.
3. 0.04 gm. al. ppt. + 1.0 cc. neg. serum + 4 cc. water.
4. 0.04 gm. al. ppt. + 5.0 cc. neg. serum.
5. 0.04 gm. al. ppt. + 5.0 cc. water.
6. 5.0 cc. positive serum.
7. 5.0 cc. negative serum.

The positive serum was obtained from a cow that had been a confirmed reactor for several years. It had an agglutinin titre of 1 : 1000. The serum was heated for 30 min. at 56° C. before the suspensions were made up. Temperatures are given in Table IV.

TABLE IV

TEMPERATURES OF GUINEA PIGS IN EXPERIMENT 6

—	10:00 a.m.	11:00 a.m.	1:30 p.m.	3:00 p.m.	4:30 p.m.	9:00 a.m.	12:00 m.	3:30 p.m.	5:00 p.m.	9:00 a.m.
1	103.0	99.2	95.8	94.8	94.0	94.0	95.8	98.4	100.2	102.0
2	103.2	100.8	98.2	95.8	95.4	101.6	102.4	103.4	102.4	102.2
3	103.0	100.0	94.0	94.0	95.2	94.0	94.0	94.0	94.0	102.0
4	103.0	98.7	94.4	94.0	94.0	94.0	94.0	94.0	94.0	dead
5	103.0	99.0	98.2	97.1	97.0	94.0	94.0	100.6	101.0	103.2
6	102.5	101.2	101.2	102.8	102.4	102.4	102.4	102.4	102.8	
7	103.8	102.0	100.0	100.0	101.2	102.4	103.4	103.0	103.2	

In this experiment positive serum was effective in preventing the temperature fall to some extent, and in more rapid recovery. Nos. 3 and 5 nearly died, but eventually recovered. The normal serum did not modify the action of the precipitate.

Experiment 7. Effect of absorbed and unabsorbed positive serum on intraperitoneal injections of precipitate. The positive serum was that employed in Experiment 6. The negative serum was obtained by pooling routine samples of blood. Both lots were heated for 30 min. at 56° C. Two cc. of packed *Br. abortus* was added to 10 cc. of positive serum. This and 10 cc. of negative serum were allowed to stand at room temperature for two days. Both serums contained 0.5% phenol as preservative. The absorbed tube was centrifuged and the serum drawn off. One guinea pig was given an intraperitoneal injection of 5.0 cc. absorbed serum and the other 5.0 cc. of untreated serum. Both serums had suspended in them 0.02 gm. of alcoholic precipitate. Fig. 2 shows the temperature reactions following the injections. The difference is very striking and is further confirmation of the specificity fo the alcoholic precipitate.

Agglutination and complement fixation tests were run on the untreated and absorbed serum and also on another tube of the same serum absorbed with alcoholic precipitate. There was not sufficient of this material to absorb a large enough quantity of serum for guinea pig injection so it was tested by the serological tests only. The results are given in Table V.

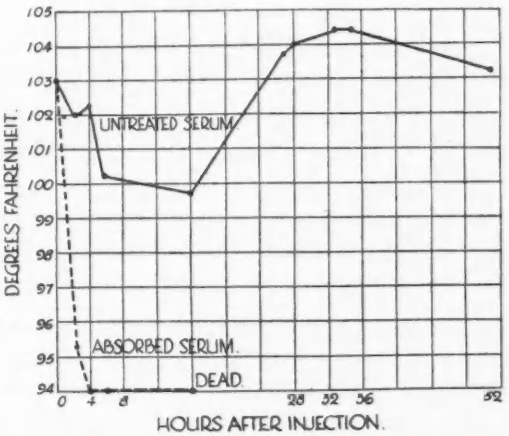

Fig. 2. Effect of absorbed and untreated immune serum on precipitate. Temperatures of guinea pigs.

This experiment showed that alcoholic precipitate was able to absorb an anti-abortus serum equally as well as the whole organism. It further showed that a serum absorbed with the whole organism afforded no protection against the alcoholic precipitate.

TABLE V

AGGLUTINATION AND COMPLEMENT FIXATION TESTS ON ABSORBED SERUMS

			1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Absorbed with al. ppt.	24 hours	Agglutination	+	—	—	—	—	—	—
		Comp. fixation	4	1	0	0	0	0	0
	48 hours	Agglutination	+	—	—	—	—	—	—
		Comp. fixation	4	0	0	0	0	0	0
Absorbed with *Br. abortus*	24 hours	Agglutination	+	—	—	—	—	—	—
		Comp. fixation	4	4	0	0	0	0	0
	48 hours	Agglutination	+	—	—	—	—	—	—
		Comp. fixation	4	4	0	0	0	0	0
Not absorbed		Agglutination	+	+	+	+	+	+	—
		Comp. fixation	4	4	4	4	4	4	4

+ = Complete agglutination; 4 = Complete fixation; 1 = 25% fixation; 0 = No fixation.

Effect of Alcoholic Precipitate on Agglutinin Production and Phagocytosis, and Result of Oral Administration

Experiment 8. Production of agglutinins by alcoholic precipitate. Four guinea pigs that had survived injections of alcoholic precipitate were tested for presence of agglutinins. Details are given in Table VI.

TABLE VI

AGGLUTININ PRODUCTION OF GUINEA PIGS INJECTED WITH ALCOHOLIC PRECIPITATE

No.	Date of injection	Dose	Date bled	Agglutination		
				1 : 25	1 : 50	1 : 100
1	May 10	0.04 gm.	May 25	—	—	—
2	May 10	0.04 gm.	May 25	—	—	—
3	May 14	0.01 gm.	May 25	—	—	—
4	April 17	0.04 gm.	May 25	+	+	—

Only one of these guinea pigs showed agglutinins at time of bleeding. This was 39 days after it had been injected. The other three were bled 15 and 11 days after injection.

Agglutinin production was clearly shown in the immunity experiments which will be described later. In one case, 12 guinea pigs, after receiving six injections of alcoholic precipitate, had agglutinin titres varying from 1 : 25 to 1 : 250.

Experiment 9. Opsono-cytophagic activity produced in rabbit by injections of alcoholic precipitate. Blood was collected from a normal rabbit, 1 cc. being drawn from the heart and placed in a tube containing 0.05 cc. of 20% sodium citrate. Following the method of Huddleson *et al*, (5) for study of the opsono-cytophagic reaction, 0.1 cc. of citrated blood and 0.1 cc. of a suspension of *Br. abortus* were mixed and incubated for 30 min. at 37° C. Smears were made and stained with Giemsa. There was no phagocytosis. The remaining citrated blood was centrifuged and the agglutination test was set up. There was no agglutination. The rabbit was then given an intraperitoneal injection of 0.04 gm. of alcoholic precipitate in 2 cc. water. At 10:00 a.m., the time of injection, the rabbit's temperature was 102.8° F. At 2:00 p.m. and 4:30 p.m. it was 104.0. The following morning the temperature was 102.6. The rabbit showed no symptoms following this injection.

One week later the rabbit was again bled. Agglutination occurred in a dilution of 1:25. There was marked phagocytosis in all the polymorphonuclear leucocytes examined. At two and three weeks after injection the agglutinin titre was still 1:25 and phagocytic activity was still marked. At one month the agglutination test was negative in 1:25 but phagocytic activity persisted. At five weeks phagocytic activity was again marked, all polymorphonuclear cells being crowded with bacteria. The agglutination test was again negative.

Experiment 10. Effect of alcoholic precipitate by mouth. Two guinea pigs were fasted overnight. In the morning each was given 0.1 gm. of alcoholic precipitate by mouth, mixed in a small quantity of water. The initial temperatures seemed to be lower than normal, which may have been due to fasting. The temperatures of these two guinea pigs are shown in Table VII.

TABLE VII

TEMPERATURES OF GUINEA PIGS RECEIVING ALCOHOLIC PRECIPITATE BY MOUTH

	10:00 a.m.	12:15 p.m.	2:10 p.m.	4:00 p.m.	9:00 a.m.	12:00 m.	2:30 p.m.	4:00 p.m.
No. 1	101.6	102.4	102.6	103.0	102.6	102.0	102.6	103.4
No. 2	101.0	101.6	102.4	102.2	101.2	102.0	101.8	102.6

These guinea pigs showed no bad effects from ingestion of 0.1 gm. alcoholic precipitate.

Immunity Experiments in Guinea Pigs with Alcoholic Precipitate

Experiment 11. Exposure to infection after six injections of alcoholic precipitate. Four guinea pigs were given six intraperitoneal injections of 0.01 gm. of Lot 4 alcoholic precipitate in 2 cc. saline over a period of two weeks. These animals and two normal guinea pigs were exposed to infection by eye with a suspension of *Br. abortus* one week after the last injection. Agglutination tests were made on the blood of these animals at the time when they were exposed, two weeks later, and then at weekly intervals until they were killed nine weeks after commencement of the experiment. There was no significant difference in post-mortem appearance of the organs of the vaccinated animals and controls and *Br. abortus* was recovered from all the spleens. Nos. 2, 3 and 4 had agglutinin titres of 1 : 500, 1 : 50 and 1 : 250, respectively, at the time they were exposed to infection. No. 1 was negative at this time and did not show agglutinins until the controls were positive. There was no evidence of protection in those animals that had been exposed to infection one week after the last of six injections of alcoholic precipitate.

Experiment 12. Exposure to infection by eye at varying intervals after six injections of alcoholic precipitate. Twenty large guinea pigs were selected. They were given an intraperitoneal injection of 0.0025 gm. of Lot 6 alcoholic precipitate in 2.0 cc. water. Temperatures were taken on the first four animals to determine the effect of the injection. There was a drop of only 1 to 1.5° F. The second injection was 0.005 gm. and again there was no disturbance of any account. The third injection was 0.01 gm. The fourth was the same as the third. The fifth was 0.015 gm. No trouble had been experienced up to this time, but following the sixth injection of 0.02 gm. all became very ill and twelve died, leaving only eight for the experiment. Injections had been given at five-day intervals with the exception of one, which was given at four days. There did not seem to be any mounting resistance against the alcoholic precipitate, as the 0.02-gm. injection was not tolerated any better than it would be by animals that had received no previous injections.

One week after the last injection the first three animals, Nos. 2, 3 and 4, were exposed to infection by eye with one drop of *Br. abortus* suspension of

a density equal to 1.0 cc. on the Gates nephelometer. Three normal guinea pigs, Nos. 21, 22 and 23, were similarly exposed at this time. Two weeks after the last injection, the next three survivors, Nos. 6, 9 and 10, were exposed in the same manner with three normal animals, Nos. 24, 25 and 26. Four weeks after the last injection the last two vaccinated animals were also exposed to infection with two normal controls, Nos. 27 and 28. These animals were bled at the time the first group was exposed to infection and then weekly until killed. The first group was killed five weeks, the second four weeks, and the third group 25 days after exposure to infection, as at that time their agglutinin titres clearly indicated that they were infected. The agglutinin titres are given in Table VIII.

TABLE VIII

AGGLUTININ TITRES OF GUINEA PIGS IN VACCINATION EXPERIMENT

No.	May 14	June 14	June 21	June 28	July 5	July 12	July 19	July 27	July 30
2	—	1 : 25	—	1 : 500	1 : 500	1 : 1000	1 : 1000		
3	—	1 : 25	1 : 50	1 : 500	1 : 500	1 : 1000	1 : 1000		
4	—	1 : 200	1 : 100	1 : 100	1 : 500	1 : 1000	1 : 1000		
6	—	—	—	1 : 25	1 : 100	1 : 500	1 : 1000		
9	—	—	—	1 : 25	1 : 250	1 : 1000	1 : 1000		
10	—	1 : 250	1 : 250	1 : 50	1 : 1000	1 : 1000	1 : 1000		
14	—	—	1 : 50	1 : 100	—	1 : 25	died		
18	—	—	1 : 50	1 : 25	—	1 : 25	1 : 100	1 : 1000	1 : 1000
21		—	—	1 : 500	1 : 500	1 : 1000	1 : 1000		
22		—	—	1 : 100	1 : 250	1 : 500	1 : 1000		
23		—	—	1 : 500	1 : 1000	1 : 1000	1 : 1000		
24			—	—	1 : 100	1 : 250	1 : 500		
25			—	—	1 : 100	1 : 100	1 : 250		
26			—	—	1 : 100	1 : 100	1 : 250		
27					—	—	1 : 50	1 : 100	1 : 100
28					—	—	1 : 100	1 : 500	1 : 1000

There was nothing in the agglutination results suggestive of protection. Four guinea pigs had agglutinins in their blood one week after the last injection. These declined and then picked up again following exposure to infection. Table IX gives post-mortem and cultural results on the vaccinated animals and their controls.

No. 14 died during the night of July 16–17 and was badly decomposed. Cultures were overgrown and the presence or absence of *Br. abortus* could not be determined. Death was probably due to the results of the alcoholic precipitate injections. We have had a certain number of guinea pigs that have lived for a considerable length of time after injections which finally proved fatal.

In this experiment, vaccinated animals and controls were alike infected. With the exception of No. 2, however, the spleens of the vaccinated animals were normal in appearance, whereas the control spleens were larger and all showed nodules. This difference was slight but it did suggest that some benefit was derived from the vaccinal injections. The weights of these

TABLE IX

POST-MORTEM AND CULTURAL RESULTS

No.	Post-mortem findings	Wt. of spleen, gm.	Cultures
2	Large spleen	1.3	+
3	Normal spleen	0.8	+
4	Normal spleen	0.75	+
6	Normal spleen	0.8	+
9	Normal spleen	0.85	+
10	Normal spleen	0.7	+
14	Died. Spleen normal. Decomposed		
18	Normal spleen	0.8	+
21	Large nodular spleen	1.6	+
22	Large nodular spleen	1.2	+
23	Large nodular spleen	2.3	+
24	Spleen enlarged. Nodular	1.1	+
25	Spleen not much enlarged but nodular	0.9	+
26	Spleen not much enlarged but nodular	0.85	+
27	Nodular spleen	0.9	+
28	Enlarged, nodular spleen	1.4	+

animals were not of value in forming an opinion, as the injèctions of precipitate had caused much loss in weight in the group.

Experiment 13. Injection of young sows with alcoholic precipitate prior to breeding. In view of the slight suggestion that some resistance may have developed in the vaccinated animals of Experiment 12, it was decided to vaccinate a group of sows with alcoholic precipitate prior to breeding, infect them at the time the males were added, and note any difference in the production of living young by these animals.

Fifteen young sows were isolated and given six injections of alcoholic precipitate (a mixture of several lots) at five-day intervals. Dosage was kept lower than in previous experiments, but in spite of this, three animals died, leaving 12 for test. Intraperitoneal injections were given in 2 cc. water, the quantity of precipitate received by each pig being approximately as follows:—

First injection	0.0023 gm.	Fourth injection	0.004 gm.
Second injection	0.0026 gm.	Fifth injection	0.003 gm.
Third injection	0.003 gm.	Sixth injection	0.003 gm.

Following the fourth injection all appeared ill and one guinea pig died. Two more died later. It is therefore obvious that maximum doses had been given. It would have been possible to have given larger doses by treating the precipitate with specific serum, but to avoid introducing other factors this was not tried. Injections were commenced June 13 and completed July 9.

On July 12, the 12 surviving vaccinated animals were divided into two lots and two groups of controls were arranged as follows:—

Group 1. Six vaccinated sows and one normal boar.

Group 2. Six normal sows and a boar.

Group 3. Six vaccinated sows and one normal boar.

Group 4. Six normal sows and a boar.

Groups 1 and 2 were exposed to infection by eye on this date with a drop of *Br. abortus* suspension equal in density to 1.0 cc. on the Gates nephelometer. The groups were bled on July 13 and the agglutination test carried out on their serum. The agglutination results are given in Table X.

Group 1 was arranged to show whether vaccinated sows that had been exposed to infection would give birth to living young. Group 2 was a control on this group to show the difference between normal and vaccinated sows that had been exposed to infection. Group 3 was to show whether vaccination with alcoholic precipitate would have any harmful effect on these sows. Group 4 was a control on Group 3, so that comparison might be made between vaccinated and unvaccinated animals which had not been exposed to infection.

TABLE X

AGGLUTINATION TESTS ON GROUPS 1, 2, 3 AND 4

Group	No.	July 13	July 20	July 27	Aug. 2	Aug. 23	Sept. 6	Oct. 29
No. 1 Vaccination and infection	1	1 : 50	1 : 50	1 : 500	1 : 500	1 : 100	1 : 500	1 : 1000
	2	1 : 100	1 : 100	1 : 500	1 : 1000	1 : 1000	1 : 1000	dead
	3	1 : 100	1 : 100	1 : 100	1 : 1000	1 : 1000	1 : 1000	1 : 1000
	4	1 : 100	1 : 100	1 : 500	1 : 1000	1 : 1000	1 : 1000	1 : 1000
	5	1 : 25	1 : 50	1 : 100	1 : 1000	1 : 1000	1 : 1000	1 : 1000
	6	1 : 100	1 : 50	1 : 50	1 : 1000	1 : 1000	1 : 1000	1 : 1000
	*7	—	—	—	—	—	1 : 25	†—
No. 2 Infection only	8	—	—	1 : 50	1 : 100	1 : 1000	1 : 500	1 : 500
	9	—	—	1 : 50	1 : 100	1 : 250	1 : 250	1 : 1000
	10	—	—	1 : 100	1 : 100	1 : 500	1 : 500	1 : 250
	11	—	—	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000
	12	—	—	1 : 100	1 : 100	1 : 500	1 : 250	dead
	13	—	—	1 : 25	1 : 100	1 : 500	1 : 250	1 : 1000
	*14	—	—	—	—	—	—	†—
No. 3 Vaccine only	15	1 : 250	1 : 250	1 : 100	1 : 100	1 : 50	1 : 50	1 : 25
	16	1 : 100	1 : 50	1 : 25	1 : 25	—	—	—
	17	1 : 50	1 : 50	—	—	—	—	—
	18	1 : 50	1 : 25	—	—	—	—	—
	19	1 : 100	1 : 100	—	—	—	—	—
	20	1 : 50	1 : 50	1 : 50	1 : 25	—	—	—
	*21	—	—	—	—	—	—	—
No. 4 Normal	22	—	Not tested					—
	23	—						—
	24	—						—
	25	—						—
	26	—						—
	27	—						—
	*28	—						—

**Boars.* †*New boars.*

The agglutinins shown by Groups 1 and 3 on July 13 were due to the vaccinal injections of alcoholic precipitate. Two weeks later the infected controls had agglutinins in their blood. These antibodies were disappearing in Group 3, vaccine only, and were increasing in Group 1, vaccination and infection. Some reaction persisted in one of the former group until the end of the experiment. From the two previous experiments it was to be expected that the vaccinated animals would become infected.

The two males in Groups 1 and 2 were killed on September 13 and were replaced by new boars. This was done to avoid handicapping these groups through the use of possibly inefficient males. Post-mortem examination, however, did not show any evidence of infection. The serum of No. 7 had an agglutinin titre of 1 : 25 and that of No. 14 was negative.

Groups 1 and 2 were killed on October 29. Each group consisted of five sows and a boar, one sow in each group having died. All the young had been aborted or died shortly after birth. At this time Group 3, vaccine only, consisted of 16 animals, the original seven, and nine young. One sow in this group aborted about 12 weeks after the last injection of alcoholic precipitate. There was no evidence that this was due to infection. Group 4, untreated, had 19 animals, the original seven, and 12 young.

Post-mortem examination showed well marked splenic lesions in the sows of Groups 1 and 2. The spleens were nodular and hemorrhagic and varied in weight from 2 to 4 gm. *Br. abortus* was recovered from all. The agglutinin titres of the serum of these sows ranged from 1 : 250 to 1 : 1000. The males had no lesions and were negative serologically and on culture. The spleens weighed 0.8 and 0.9 gm. None of Group 1, vaccination and infection, was pregnant but two of the unvaccinated group exposed to infection were in early pregnancy. *Br. abortus* was not recovered from the placentas of these sows.

In this experiment, six intraperitoneal injections of alcoholic precipitate prior to breeding did not increase the breeding efficiency of guinea pigs that were subsequently exposed to infection. One of the control group that received only injections of precipitate aborted about 12 weeks after the last injection. The cause was not determined.

Summary

The specificity of alcoholic precipitate was shown *in vivo* by the fact that it was neutralized by injection of immune serum but was not affected by normal serum.

Immune serum absorbed with *Br. abortus* showed no protective action against alcoholic precipitate, the protective substance having been removed by absorption.

Alcoholic precipitate was as efficient as whole organisms in removing antibodies, as judged by the agglutination and complement fixation tests.

Fresh rabbit blood and serum caused a drop in temperature in guinea pigs treated by the intraperitoneal route. This serum was shown to be hemolytic for guinea-pig red cells *in vitro*.

Alcoholic precipitate of *Br. abortus* gave rise to agglutinins in the blood of guinea pigs and a rabbit. Where only one injection was given, agglutinin production was less common.

One injection of alcoholic precipitate in a rabbit produced very marked opsono-cytophagic activity against *Br. abortus* one week after injection and this persisted for the five weeks the animal was under test. The agglutinin titre was 1 : 25 one week after injection and disappeared the fourth week.

One-tenth gram of alcoholic precipitate by mouth did not produce any temperature reaction or ill effects in two guinea pigs that had been fasted overnight.

In the first immunity experiment four guinea pigs were given six intraperitoneal injections of 0.01 gm. of precipitate over a period of two weeks. These and two controls were exposed to infection by eye one week after the last injection. There was no indication of resistance to infection in these animals.

In the second experiment guinea pigs were given six intraperitoneal injections of alcoholic precipitate ranging from 0.0025 gm. to 0.02 gm. at five-day intervals. The last dose proved to be too large, causing a 60% mortality. The remaining animals and normal controls were exposed to infection by eye in three groups, one, two, and four weeks after the last injection of precipitate. All became infected as shown by agglutinin titres and spleen cultures but seven out of eight of the vaccinated animals had spleens which were normal in appearance and weight, whereas all the unvaccinated control spleens were distinctly nodular and those from the first group, which had been infected five weeks, were large. The difference in size was not as marked in those animals that had been held shorter periods.

In the third experiment, guinea pigs were given six intraperitoneal injections of alcoholic precipitate prior to breeding. Four groups consisting of six sows and one boar were used. The six normal sows had 12 healthy young. Six sows that were injected with alcoholic precipitate, but not exposed to infection, had nine young. There were no young in the vaccinated and unvaccinated groups that had been exposed to infection, but on post-mortem examination two of the latter group were found to be in early pregnancy. *Br. abortus* was not recovered from the placentas. One sow in each of the infected groups died following parturition. All showed well-marked spleen lesions which yielded *Br. abortus* on culture. There was no significant difference between the sows of these two groups. All showed lesions, were positive by the agglutination test and by cultural methods. Agglutinins disappeared from the blood of five of the animals that had been injected with alcoholic precipitate but not exposed to infection. One still showed a slight reaction at the conclusion of the experiment. One of the sows in this group aborted. There was no evidence that this was due to infection.

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